Appendix A: Infringement Claim Chart for U.S. Patent No. 12,168,797 and QX600

Bio-Rad's QX600 Droplet Digital PCR (ddPCR) System ("QX600") infringes Claims 1–2, 5–11, 13–15, 18–19, and 21 of U.S. Patent No. 12,168,797 ("the '797 Patent"). Reference to the QX600 will be understood to refer to all versions of the system, e.g., the QX600 Droplet Digital PCR System and the QX600 AutoDG Droplet Digital PCR System, including all the various additional devices, software, accessories, and/or components used in connection with any version.

As non-limiting examples, Caltech identifies where and how the QX600 comprises each and every element of the below claimed systems. Elements from other charts included with these Infringement Contentions may be used in combination with the below to meet elements of the claimed systems, and Caltech incorporates all such other charts here by reference.

On information and belief, the QX600 is used with other devices, accessories, and components to meet the elements of claimed systems of the '797 Patent. *See* CT0481927 at CT0481928–CT0481944 and CT0481945 at CT0481946–CT0481962. A list of non-limiting examples of such devices, accessories, and/or components include:

- The QX600 Droplet Reader;
- Software, such as the QX Manager Software (Premium and/or Standard Editions);
- A droplet generator, such as the Bio-Rad Automated Droplet Generator and/or QX200 Droplet Generator;
- A thermal cycler, such as the C1000 Touch Thermal Cycler with 96-Deepwell Reaction Module and/or PTC Tempo Deepwell Thermal Cycler;
- A plate sealer, such as the PX1 PCR Plate Sealer;
- ddPCR kits and reagents, such as
    - PrimePCR ddPCR Assays,
    - ddPCR Multiplex Supermix,
    - ddPCR Supermixes for Probes (No dUTP),
    - ddPCR Supermix for Probes,
    - One-Step RT-ddPCR Advanced Kit for Probes,
    - ddPCR Buffer Control for Probes,
    - Residual DNA Quantification Supermixes,
    - QX200 ddPCR EvaGreen Supermix,
    - QX200 Buffer Control for EvaGreen, and
    - ddPCR Library Quantification Kit for Illumina TruSeq;
- Consumables, such as
    - ddPCR 96-Well Plates,

    - Droplet Generation Oil for Probes,
    - QX200 Droplet Generation Oil for EvaGreen,
    - Droplet Reader Oil,
    - DG8 Cartridges and DG8 Gaskets,
    - DG8 Cartridge Holders,
    - pierceable foil such as the PCR Plate Heat Seal,
    - DG32 Cartridges for AutoDG,
    - Pipet Tips for AutoDG,
    - Pipet Tip Waste Bin for AutoDG;
- PrimePCR Primers, Assays and Arrays;
- Each of the assays identified in these Infringement Contentions; and/or
- Any other devices, software, accessories, and components that Bio-Rad instructs or encourages its customers and end users to use with the QX600.

Bio-Rad directly infringes the Asserted Claims by making, using, offering to sell, and/or selling the QX600 alone and/or with the above-listed devices, software, accessories, and/or components within the United States or by importing the QX600 alone and/or with the above-listed devices, software, accessories, and/or components to the United States. The QX600 alone and/or with the above-listed devices, software, accessories, and/or components meets each and every element of the below claimed systems. By way of non-limiting example, Bio-Rad offers to sell the QX600 in connection with (but not exclusively with) the accompanying QX Manager Software to meet each and every element of the Asserted Claims.

Bio-Rad also indirectly infringes the Asserted Claims by providing the QX600 alone and/or with the above-listed devices, software, accessories, and/or components. Bio-Rad induces customers and/or end users to infringe by providing the QX600 to such customers and/or end users, or by providing the QX600 in combination with the above-listed devices, software, accessories, and/or components to such customers and/or end users, for use in a way that meets every element of the Asserted Claims as described below and throughout these Infringement Contentions in violation of 35 U.S.C. § 271(b). By way of non-limiting example, Bio-Rad provides such customers and/or end users with the QX600 and the accompanying QX Manager Software for use in a way that meets each and every element of the Asserted Claims.

Bio-Rad also contributes to the infringement of customers and/or end-users by providing the QX600 to such customers and/or end users, or by providing the QX600 in combination with the above-listed devices, software, accessories, and/or components to such customers and/or end users, and such customers and/or end-users then use the QX600 alone and/or with the above-listed devices, software, accessories, and/or components to meet every element of the Asserted Claims as described below and throughout these

BIO-RAD EX.1009.002

Infringement Contentions in violation of 35 U.S.C. § 271(c). By way of non-limiting example, such customers and/or end users use the QX600 and the accompanying QX Manager Software for use in a way that meets each and every element of the Asserted Claims.

On information and belief, Bio-Rad also supplies or causes to be supplied in or from the United States all or a substantial portion of the QX600 and/or the above-listed devices, software, accessories, and/or components such that Bio-Rad induces the infringement of customers or end users outside the United States in violation of 35 U.S.C. § 271(f)(1). For like reasons as described above, Bio-Rad contributes to the infringement of customers or end users outside the United States in violation of 35 U.S.C. § 271(f)(2).

Caltech alleges direct and indirect infringement both literally and under the doctrine of equivalents.

As non-limiting examples, Caltech identifies where and how the QX600 meets each and every element of the below claimed systems.

Claims	Exemplary Disclosure of Accused Product
[1.pre] 1. A system comprising:	To the extent the preamble is found to be limiting, the QX600 is a system comprising each and every element below, both literally and under the doctrine of equivalents. For example, the QX600 is a "system" comprising multiple components, each of which is itself a "system," including at least a droplet generator (QX200 Droplet Generator or AutoDG), QX600 Droplet Reader, thermal cycler, and a computer, as well as software and consumables. *See, e.g.*:

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Claims	Exemplary Disclosure of Accused Product
	# Appendix E Ordering Information This appendix contains descriptions and catalog numbers for new or replacement instruments, accessories, and consumables for Bio-Rad's ddPCR products, including your QX600 Droplet Reader. **ddPCR System and Instrument Packages** Table 41 contains information on Bio-Rad Laboratories, Inc. Droplet Digital PCR systems, as well as individual ddPCR instruments and included accessories. **Table 41. ddPCR System and Instrument Packages** | Product	Description	Catalog Number
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QX200 Droplet Digital PCR System	Includes the QX200 Droplet Generator, QX200 Droplet Reader, computer, software, and consumables	1864001
QX600 Droplet Digital PCR System	Includes the QX200 Droplet Generator, QX600 Droplet Reader, computer, software, and consumables	17007769
QX600 AutoDG™ Droplet Digital PCR System	Includes the Automated Droplet Generator (AutoDG), QX600 Droplet Reader, computer, software, and consumables	17008371
QX200 Droplet Generator	Includes the droplet generator (1), cartridges and gaskets (24 each), cartridge holders (2), and power cord (1)	1864002
QX200 Droplet Reader	Includes the droplet reader (1), plate holders (2), USB cable (1), and power cord (1)	1864003
QX600 Droplet Reader	Includes the droplet reader (1), plate holders (2), USB cable (1), and power cord (1)	12013328
Automated Droplet Generator	Includes the automated droplet generator (1), cooling block accessory (1), oil purge reservoir (1), and power cord (1)	1864101
C1000 Thermal Cycler	Thermal cycler with 384-well reaction module	1851384	 CT0479068 at CT0479276

BIO-RAD EX.1009.004

Claims	Exemplary Disclosure of Accused Product
	QX600 Droplet Reader Accessories Table 42 contains information on accessories for the QX600 Droplet Reader. **Table 43. QX600 Droplet Reader Accessories** Product / Description / Catalog Number Computer — Computer that connects to the QX600 Droplet Reader for data collection and analysis — 12017458 USB cable and power cord — Cable connecting the computer to the instrument and power cord connecting the instrument to the power source **Note:** Contact Bio-Rad Technical Support for replacement information. — Included Droplet reader plate holders (2) — Used to position the 96-well plate in the droplet reader plate compartment — 12006834 CT0479068 at CT0479278 According to at least the examples provided above, and as explained in further detail for each and every element below, the QX600 and QX600 Droplet Reader are systems comprising each and every element of independent claim 1, both literally and under the doctrine of equivalents.

BIO-RAD EX.1009.005

Claims	Exemplary Disclosure of Accused Product
[1.a] a sample chamber configured to house a sample and analyte-specific reagent mixtures of analyte-specific hybridization probes and multiple fluorophores;	The QX600 comprises a sample chamber configured to house a sample and analyte-specific reagent mixtures of analyte-specific hybridization probes and multiple fluorophores, both literally and under the doctrine of equivalents. For example, the QX600 ddPCR workflow includes combining a sample (e.g., DNA or RNA) with an analyte-specific reagent mixture (e.g., primers, probes, and Bio-Rad ddPCR supermix). *See, e.g.*: **ddPCR Workflow** The ddPCR process adheres to the following workflow: ■ You prepare your samples for PCR by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermix. ■ A droplet generator fractionates a sample into~20,000 nanoliter-sized droplets with target and background DNA distributed randomly into the droplets during the partitioning process. ■ Following droplet generation, the droplets are run through a thermal cycler, which performs PCR amplification of the nucleic acid target in each individual droplet. ■ A droplet reader reads each droplet to determine the fraction of positive droplets in the original sample and uses Poisson statistical formulas to determine the absolute quantity. **Note:** Positive droplets containing at least one copy of the target DNA molecule exhibit increased fluorescence compared to negative droplets. CT0479068 at CT0479089 The analyte-specific reagent mixtures (e.g. primers, probes, and Bio-Rad ddPCR supermix) include

BIO-RAD EX.1009.006

Claims	Exemplary Disclosure of Accused Product
	certain PCR supermixes for probe-based assays, including hydrolysis probe-based assays. *See, e.g.*:

Supermixes

QX Manager Software offers five PCR supermixes. Each supermix is optimized to deliver maximum PCR efficiency and sensitivity for the amplification and detection of DNA and RNA targets.

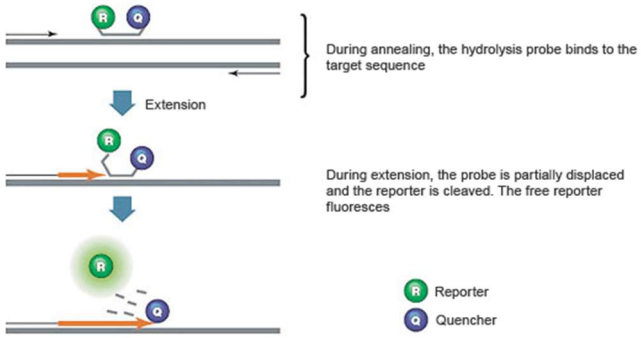
Important: Use of unapproved supermixes can harm the instrument and void the warranty.

ddPCR Supermix	Description
Multiplex Supermix	For use in nucleic acid sample preparations to amplify and detect DNA targets in probe-based experiments involving multiple targets. **Note:** Bio-Rad recommends using ddPCR Multiplex Supermix for Cy5 and Cy5.5 probes assays.
Supermix for Probes (No dUTP)	For use in nucleic acid sample preparations, with sensitivity for the amplification and detection of DNA targets using hydrolysis probe-based assays.
Supermix for Probes	For use in sample preparation with uracil N-glucosylase (UNG) decontamination protocols to prevent the reamplification of carryover PCR products between experiments.
Supermix for Residual DNA Quantification	For use in residual DNA detection.
One-Step RT Advanced Kit for Probe	For use in absolute quantification of target RNA molecules.
EvaGreen® Supermix	For use in gene expression.

BIO-RAD EX.1009.007

Claims	Exemplary Disclosure of Accused Product
	CT0479068 at CT0479138 The analyte-specific reagent mixture includes analyte-specific hybridization probes and multiple fluorophores. Probes in Bio-Rad's probe-based assays include sequence-specific molecules attached to fluorescent reporter molecules. *See, e.g.*:

BIO-RAD EX.1009.008

Claims	Exemplary Disclosure of Accused Product
	Hydrolysis (TaqMan) Probes Hydrolysis assays (TaqMan or 5' nuclease assays) include a sequence-specific, fluorescently labeled oligonucleotide probe in addition to a sequence-specific PCR primer. Hydrolysis assays exploit the 5' → 3' exonuclease activity of certain thermostable polymerases such as *Taq* or *Tth*. The hydrolysis probe is labeled with a fluorescent reporter at the 5' end and a quencher at the 3' end. Commonly used fluorescent reporter-quencher pairs are fluorescein (FAM), which emits green fluorescence, and Black Hole Quencher 1 dye.  **Fig. 2. Hydrolysis probes are hydrolyzed by *Taq* polymerase.** When the hydrolysis probe is intact, the fluorescence of the reporter is quenched due to its proximity to the quencher (Figure 2). The amplification reaction includes a combined annealing/extension step during which the probe hybridizes to the target, and the dsDNA-specific 5' → 3' exonuclease activity of *Taq* or *Tth* cleaves off the reporter. As a result, the reporter is separated from the quencher, resulting in a fluorescence signal that is proportional to the amount of amplified product in the sample. The main advantages of using hydrolysis probes are high specificity, a high signal-to-noise ratio, and the ability to perform multiplex reactions. The disadvantages are that the initial cost of the probe may be high, and the assay design may not be trivial. CT0481963 at CT0481965–CT0481966 The analyte-specific reagent mixture is combined with the sample (e.g., DNA or RNA) and placed in

BIO-RAD EX.1009.009

Claims	Exemplary Disclosure of Accused Product
	a tube or ddPCR 96-Well Plate. *See, e.g.*:

Preparing the Sample

This section contains suggested best practices and procedures for preparing the sample for the droplet generation run. Note the following general recommendations for best practices in preparing the PCR reaction.

- Combine ddPCR supermix, 20x primers and probe, and DNA sample according to the instructions in the supermix product insert.
- Use standard lab precautions to avoid contamination of the reaction mix and sample. Wear gloves, work in a clean area (such as a PCR hood) and use clean pipets and low protein binding tubes.
- Use only the PCR supermixes recommended in Appendix C, Ordering Information.
- Approved supermixes contain reagents required for droplet generation. Follow instructions in the product inserts to prepare the samples for droplet generation.
- Using unapproved supermixes can harm the instrument and void the warranty.
- Ensure the supermix concentration is 1x in final reaction.
- Prepare the sample in low-bind Eppendorf tubes or ddPCR 96-Well Plate (catalog #12001925).
- The concentration of intact human genomic DNA should be less than 66 ng per 20 µl reaction. If using higher concentrations, digest DNA with a restriction endonuclease that does not cut target or reference amplicons.

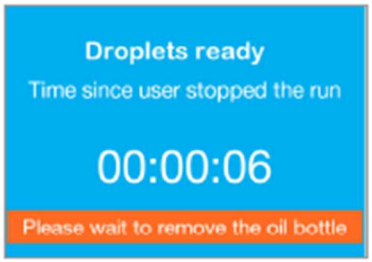
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After combining the sample (e.g., DNA or RNA) with the analyte-specific reagent mixtures (e.g.

BIO-RAD EX.1009.010

Claims	Exemplary Disclosure of Accused Product
	primers, probes, and Bio-Rad ddPCR supermix), a droplet generator fractionates the combination into droplets within the tubes or 96-well plate. *See e.g.*: **ddPCR Workflow** The ddPCR process adheres to the following workflow: ■ You prepare your samples for PCR by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermix. ■ A droplet generator fractionates a sample into~20,000 nanoliter-sized droplets with target and background DNA distributed randomly into the droplets during the partitioning process. ■ Following droplet generation, the droplets are run through a thermal cycler, which performs PCR amplification of the nucleic acid target in each individual droplet. ■ A droplet reader reads each droplet to determine the fraction of positive droplets in the original sample and uses Poisson statistical formulas to determine the absolute quantity. **Note:** Positive droplets containing at least one copy of the target DNA molecule exhibit increased fluorescence compared to negative droplets. CT0479068 at CT0479089

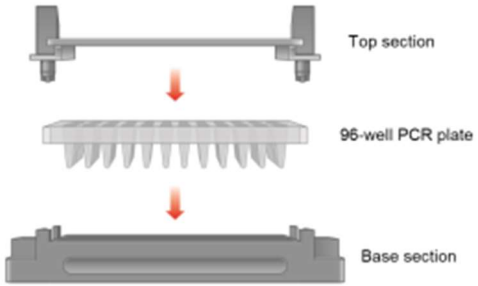
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Claims	Exemplary Disclosure of Accused Product
	When the run is finished, the following blue pulsing screen appears and the timer begins counting forward to show the time elapsed since the run concluded.  Messages to the user appear below the timer. **Important:** Do not remove the oil bottle until the unused oils are returned and the highlighted message disappears. 3. When the door on the instrument unlocks, remove the droplet plate from the instrument. 4. Remove and discard used or empty single-use consumables, such as cartridges, pipet tips, and plates. 5. Seal the plate for thermal cycling and droplet reading. For information, refer to the user guides for sealing, thermal cycling, and droplet reading instruments. CT0479286 at CT0479338 The 96-well plate containing the droplets is then inserted into the QX600 Droplet Reader for analysis: *See, e.g.*:

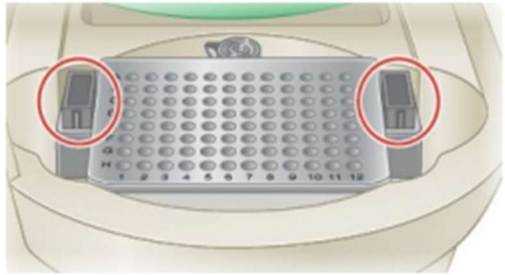
BIO-RAD EX.1009.012

Claims	Exemplary Disclosure of Accused Product
	ddPCR Workflow The ddPCR process adheres to the following workflow: ■ You prepare your samples for PCR by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermix. ■ A droplet generator fractionates a sample into~20,000 nanoliter-sized droplets with target and background DNA distributed randomly into the droplets during the partitioning process. ■ Following droplet generation, the droplets are run through a thermal cycler, which performs PCR amplification of the nucleic acid target in each individual droplet. ■ A droplet reader reads each droplet to determine the fraction of positive droplets in the original sample and uses Poisson statistical formulas to determine the absolute quantity. **Note:** Positive droplets containing at least one copy of the target DNA molecule exhibit increased fluorescence compared to negative droplets. CT0479068 at CT0479089

BIO-RAD EX.1009.013

Claims	Exemplary Disclosure of Accused Product
	Assembling a Plate The QX600 Droplet Reader accepts a solid 96-well plate that fits into a secure holder. You must assemble the plate components before you can insert the plate into the QX600 Droplet Reader.Three separate components comprise the plate: ■ A base section to hold the plate ■ The plate itself ■ A top section to secure the plate  CT0479068 at CT0479114

BIO-RAD EX.1009.014

Claims	Exemplary Disclosure of Accused Product
	Important: Make sure the tabs on the plate holder are securely clamped and the plate lays flat. Otherwise, the sampling needle can strike the lid and damage the instrument.  3. Press the button again to close the lid. CT0479068 at CT0479115 According to at least the examples above, the QX600 comprises a sample chamber (e.g., the volume within the QX600 Droplet Reader within which the 96-well plate is inserted) configured to house a sample (e.g., DNA or RNA) and analyte-specific reagent mixtures of analyte-specific hybridization probes and multiple fluorophores (e.g., primers, probes, and Bio-Rad ddPCR supermix mixed together), both literally and under the doctrine of equivalents.

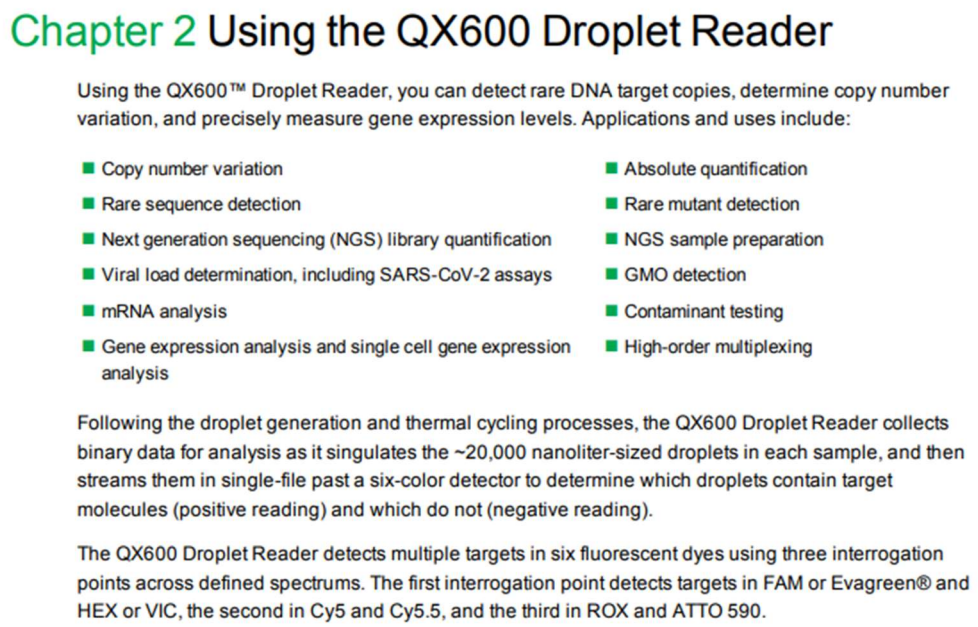
BIO-RAD EX.1009.015

Claims	Exemplary Disclosure of Accused Product
[1.b] a multi-channel detector to detect:	The QX600 comprises a multi-channel detector, both literally and under the doctrine of equivalents. The QX600 Droplet Reader within the QX600 comprises a multi-channel detector (e.g., a six-color detector). *See, e.g.*: Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). CT0479068 at CT0479090 According to at least the examples above, the QX600 comprises a multi-channel detector (e.g., a six-

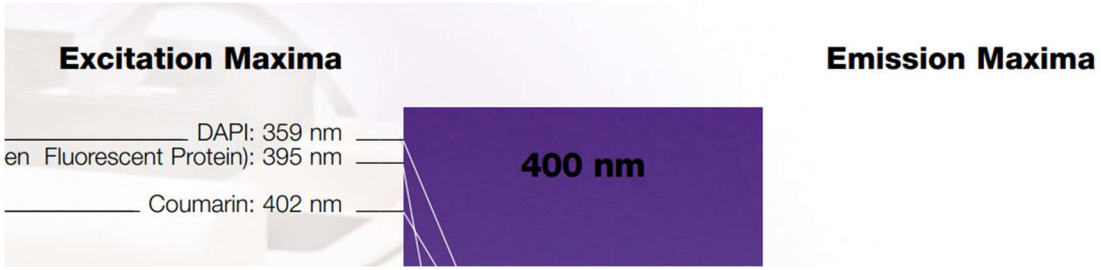
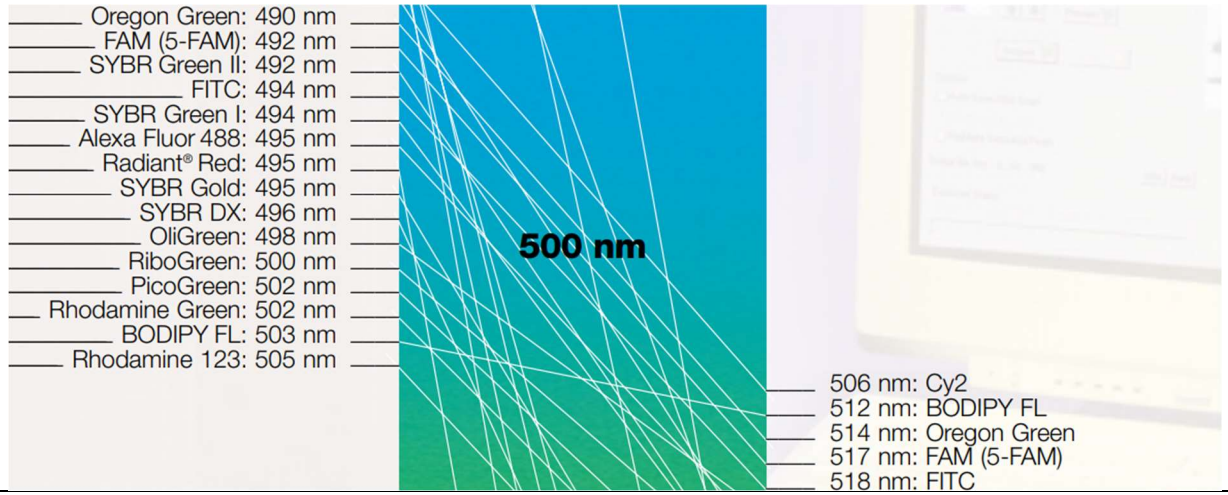
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Claims	Exemplary Disclosure of Accused Product
	color detector), both literally and under the doctrine of equivalents.
[1.b.1] a first electromagnetic signal at a first wavelength from the sample chamber, the first electromagnetic signal generated by excitement of a first fluorophore of the multiple fluorophores;	The multi-channel detector detects a first electromagnetic signal at a first wavelength from the sample chamber, the first electromagnetic signal generated by excitement of a first fluorophore of the multiple fluorophores, both literally and under the doctrine of equivalents. For example, the multi-channel detector (e.g., the QX600 Droplet Reader's six-color detector) detects electromagnetic signals in a FAM fluorophore emission wavelength after interrogating the droplet in a defined spectrum around the FAM fluorophore's excitation wavelength. *See, e.g.*:

BIO-RAD EX.1009.017

Claims	Exemplary Disclosure of Accused Product
	 Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 The FAM fluorophore emits a first electromagnetic signal in a FAM fluorophore emission wavelength (e.g., 517 nm) upon excitation (e.g., interrogation) by the QX600 Droplet Reader. *See, e.g.*:

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Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364 

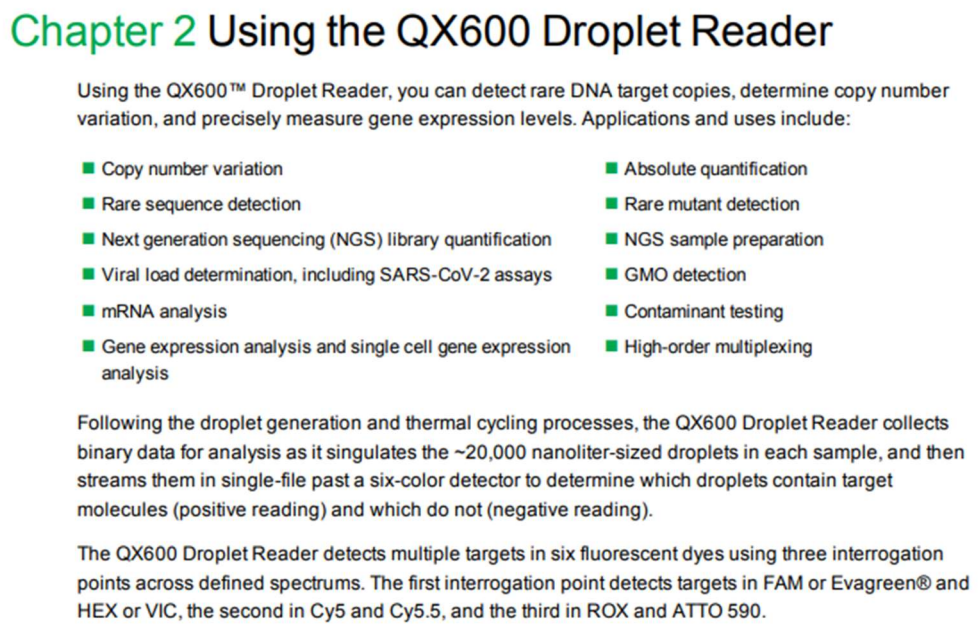
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Claims	Exemplary Disclosure of Accused Product
	CT0479364 at CT0479364 The FAM fluorophore is one of multiple fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590) that the QX600 Droplet Reader can detect after excitation (e.g., interrogation) at the given fluorophore excitation wavelength. *See, e.g.*: Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 According to at least the examples above, the multi-channel detector (e.g., the QX600 Droplet Reader's six-color detector) detects a first electromagnetic signal at a first wavelength (e.g., 517 nm)

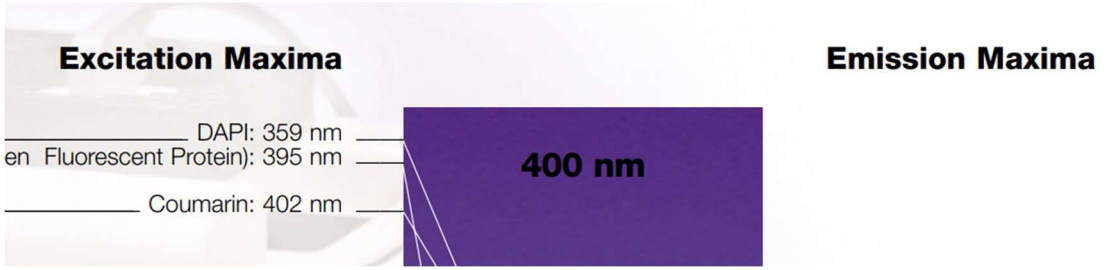
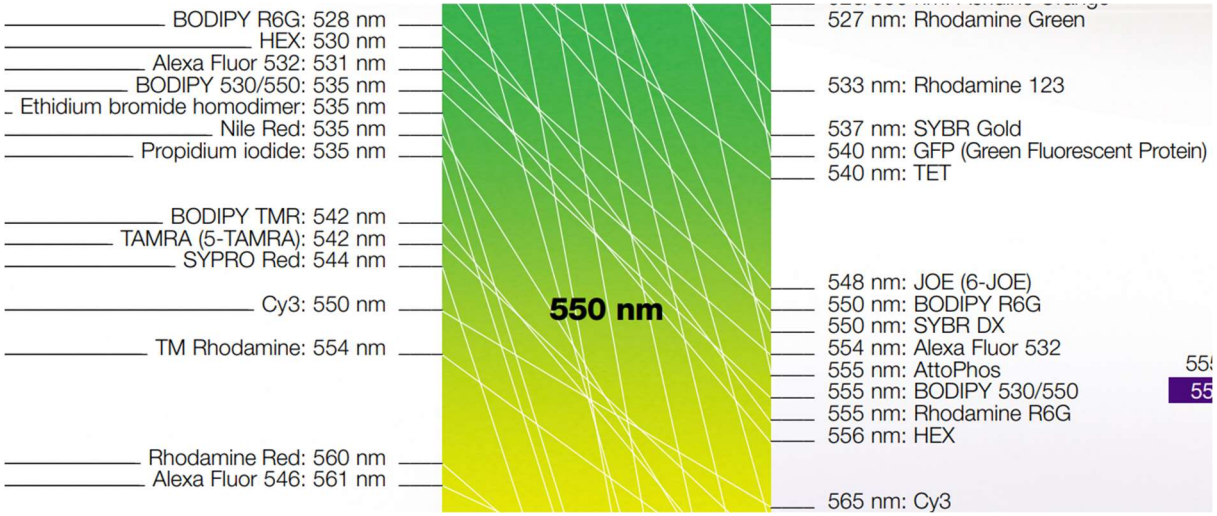
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Claims	Exemplary Disclosure of Accused Product
	from the sample chamber, the first electromagnetic signal generated by excitement (e.g., interrogation) of a first fluorophore (e.g., the FAM fluorophore) of the multiple fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590), both literally and under the doctrine of equivalents. While FAM is designated here as the example first fluorophore and its emission wavelength is designated as the first wavelength, the first fluorophore can be any one of the FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590 fluorophores that is not designated as the second, third, or fourth fluorophores, and the first wavelength is the emission wavelength of that fluorophore.
[1.b.2] a second electromagnetic signal at a second wavelength from the sample chamber, the second electromagnetic signal generated by excitement of a second fluorophore of the multiple fluorophores;	The multi-channel detector detects a second electromagnetic signal at a second wavelength from the sample chamber, the second electromagnetic signal generated by excitement of a second fluorophore of the multiple fluorophores, both literally and under the doctrine of equivalents. For example, the multi-channel detector (e.g., the QX600 Droplet Reader's six-color detector) detects electromagnetic signals in a HEX fluorophore emission wavelength after interrogating the droplet in a defined spectrum around the HEX fluorophore's excitation wavelength. *See, e.g.*:

BIO-RAD EX.1009.021

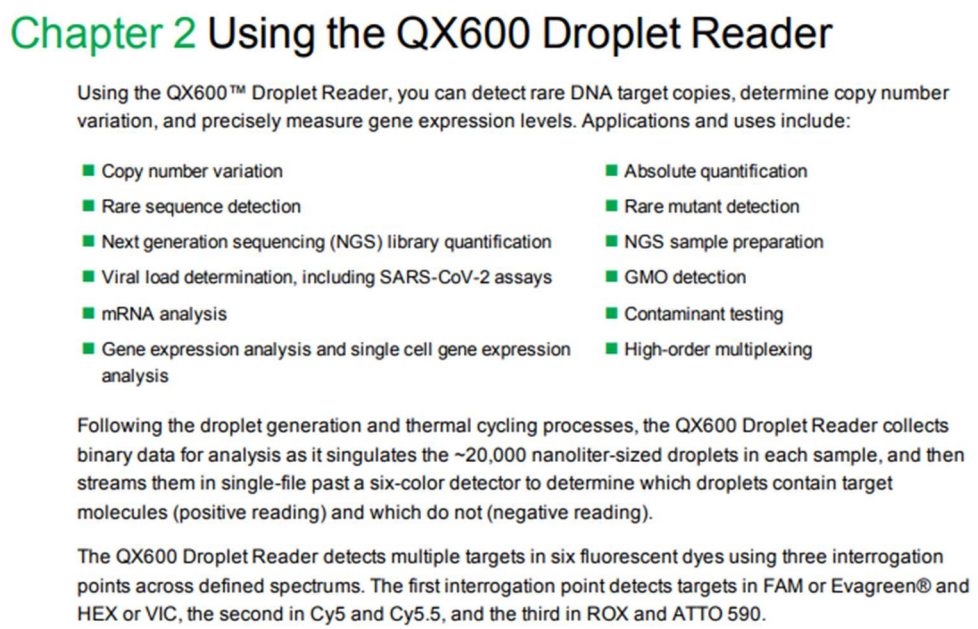
Claims	Exemplary Disclosure of Accused Product
	 CT0479068 at CT0479090 The HEX fluorophore emits a second electromagnetic signal in a HEX fluorophore emission wavelength (e.g., 556 nm), upon excitation (e.g., interrogation) by the QX600 Droplet Reader. *See, e.g.*:

BIO-RAD EX.1009.022

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364  CT0479364 at CT0479364 The HEX fluorophore is one of multiple fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and

BIO-RAD EX.1009.023

Claims	Exemplary Disclosure of Accused Product
	ATTO 590) that the QX600 Droplet Reader can detect after excitation (e.g., interrogation) at the given fluorophore excitation wavelength. *See, e.g.*:



Chapter 2 Using the QX600 Droplet Reader

Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include:

- Copy number variation
- Rare sequence detection
- Next generation sequencing (NGS) library quantification
- Viral load determination, including SARS-CoV-2 assays
- mRNA analysis
- Gene expression analysis and single cell gene expression analysis
- Absolute quantification
- Rare mutant detection
- NGS sample preparation
- GMO detection
- Contaminant testing
- High-order multiplexing

Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading).

The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590.

CT0479068 at CT0479090

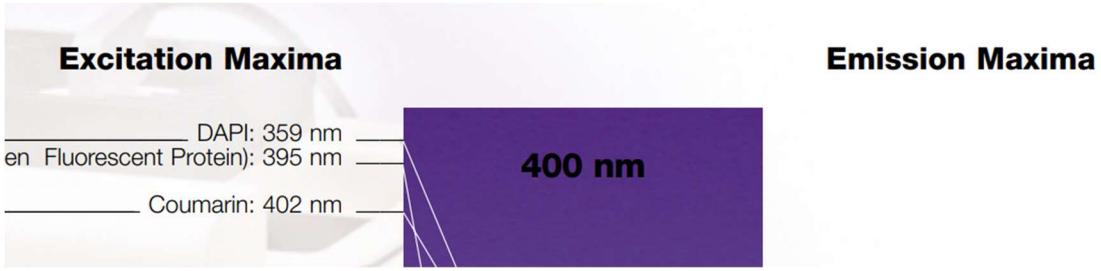
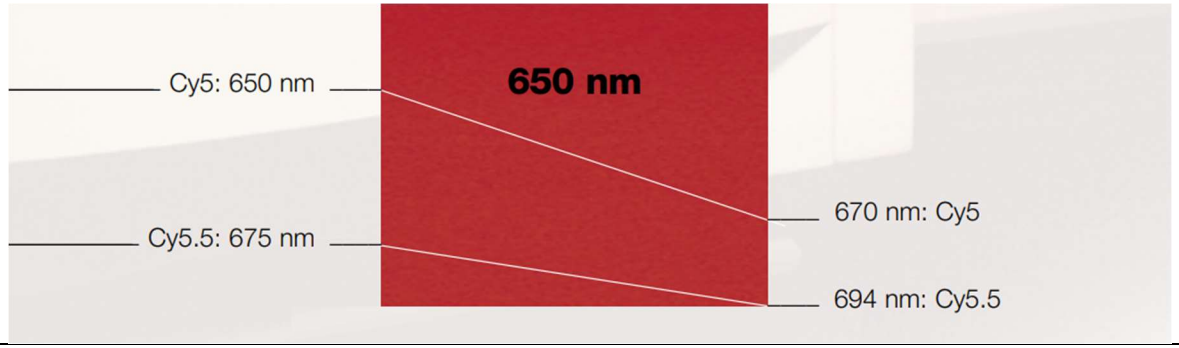
According to at least the examples above, the multi-channel detector (e.g., the QX600 Droplet Reader's six-color detector) detects a second electromagnetic signal at a second wavelength (e.g., 556 nm) from the sample chamber, the second electromagnetic signal generated by excitement (e.g., interrogation) of a second fluorophore (e.g., the HEX fluorophore) of the multiple fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590), both literally and under the doctrine of equivalents.

While HEX is designated here as the example second fluorophore and its emission wavelength is

BIO-RAD EX.1009.024

Claims	Exemplary Disclosure of Accused Product
	designated as the second wavelength, the second fluorophore can be any one of the FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590 fluorophores that is not designated as the first, third, or fourth fluorophores, and the second wavelength is the emission wavelength of that fluorophore.
[1.b.3] a third electromagnetic signal at a third wavelength from the sample chamber, the third electromagnetic signal generated by excitement of a third fluorophore of the multiple fluorophores;	The multi-channel detector detects a third electromagnetic signal at a third wavelength from the sample chamber, the third electromagnetic signal generated by excitement of a third fluorophore of the multiple fluorophores, both literally and under the doctrine of equivalents. For example, the multi-channel detector (e.g., the QX600 Droplet Reader's six-color detector) detects electromagnetic signals in a Cy5 fluorophore emission wavelength after interrogating the droplet in a defined spectrum around the Cy5 fluorophore's excitation wavelength. *See, e.g.*: Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590.

BIO-RAD EX.1009.025

Claims	Exemplary Disclosure of Accused Product
	CT0479068 at CT0479090 The Cy5 fluorophore emits a third electromagnetic signal in a Cy5 fluorophore emission wavelength (e.g., 670 nm), upon excitation (e.g., interrogation) by the QX600 Droplet Reader. *See, e.g.*:  CT0479364 at CT0479364 

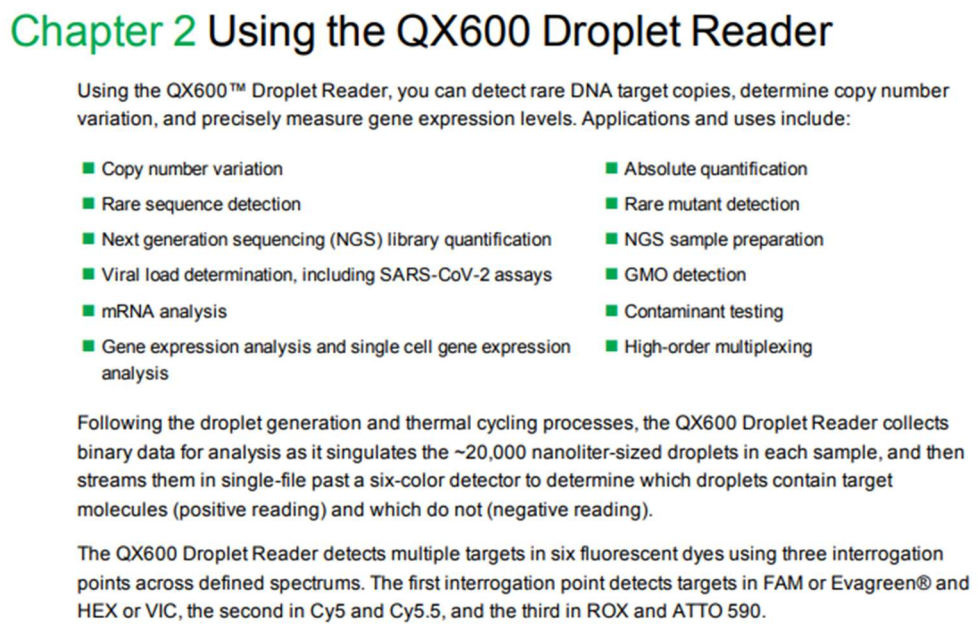
BIO-RAD EX.1009.026

Claims	Exemplary Disclosure of Accused Product
	CT0479364 at CT0479364 The Cy5 fluorophore is one of multiple fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590) that the QX600 Droplet Reader can detect after excitation (e.g., interrogation) at the given fluorophore excitation wavelength. *See, e.g.*: Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 According to at least the examples above, the multi-channel detector (e.g., the QX600 Droplet

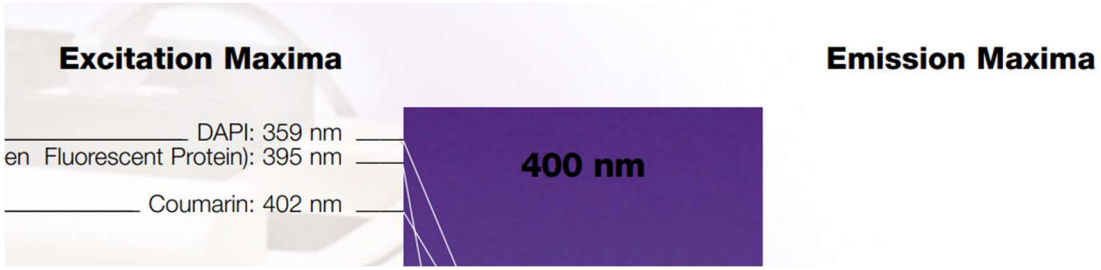
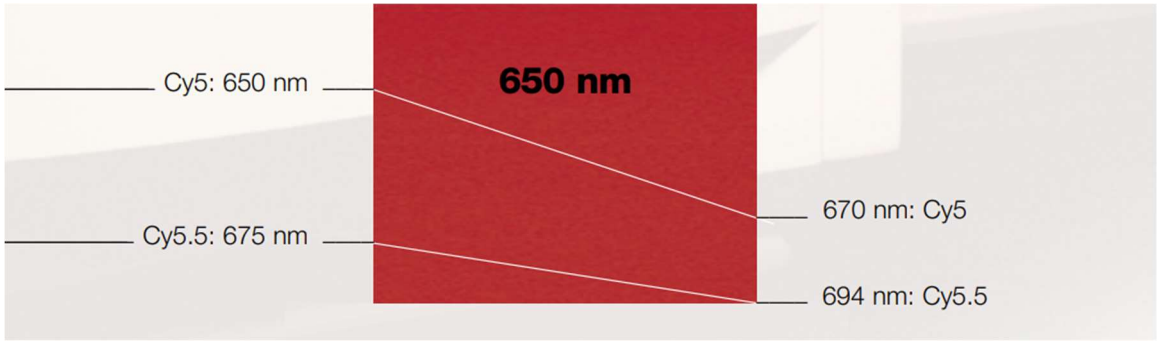
BIO-RAD EX.1009.027

Claims	Exemplary Disclosure of Accused Product
	Reader's six-color detector) detects a third electromagnetic signal at a third wavelength (e.g., 670 nm) from the sample chamber, the third electromagnetic signal generated by excitement (e.g., interrogation) of a third fluorophore (e.g., the Cy5 fluorophore) of the multiple fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590), both literally and under the doctrine of equivalents. While Cy5 is designated here as the example third fluorophore and its emission wavelength is designated as the third wavelength, the third fluorophore can be any one of the FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590 fluorophores that is not designated as the first, second, or fourth fluorophores, and the third wavelength is the emission wavelength of that fluorophore.
[1.b.4] a fourth electromagnetic signal at a fourth wavelength from the sample chamber, the fourth electromagnetic signal generated by excitement of a fourth fluorophore of the multiple fluorophores;	The multi-channel detector detects a fourth electromagnetic signal at a fourth wavelength from the sample chamber, the fourth electromagnetic signal generated by excitement of a fourth fluorophore of the multiple fluorophores, both literally and under the doctrine of equivalents. For example, the multi-channel detector (e.g., the QX600 Droplet Reader's six-color detector) detects electromagnetic signals in a Cy5.5 fluorophore emission wavelength after interrogating the droplet in a defined spectrum around the Cy5.5 fluorophore's excitation wavelength. *See, e.g.*:

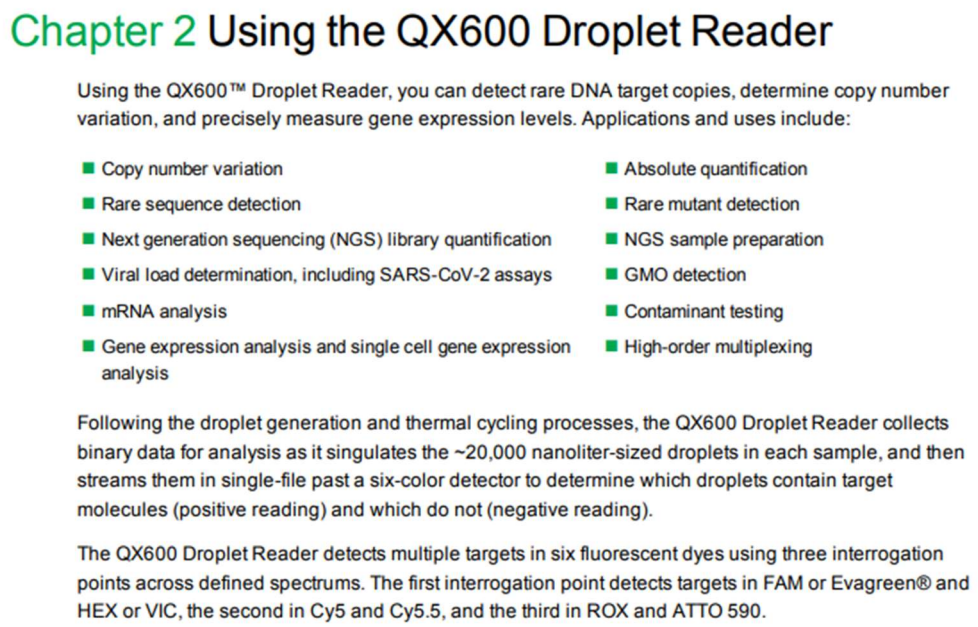
BIO-RAD EX.1009.028

Claims	Exemplary Disclosure of Accused Product
	 CT0479068 at CT0479090 The Cy5.5 fluorophore emits a fourth electromagnetic signal in a Cy5.5 fluorophore emission wavelength (e.g., 694 nm), upon excitation (e.g., interrogation) by the QX600 Droplet Reader. *See, e.g.*:

BIO-RAD EX.1009.029

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364  CT0479364 at CT0479364 The Cy5.5 fluorophore is one of multiple fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590) that the QX600 Droplet Reader can detect after excitation (e.g., interrogation) at the given fluorophore excitation wavelength. *See, e.g.*:

BIO-RAD EX.1009.030

Claims	Exemplary Disclosure of Accused Product
	 Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 According to at least the examples above, the multi-channel detector (e.g., the QX600 Droplet Reader's six-color detector) detects a fourth electromagnetic signal at a fourth wavelength (e.g., 694 nm) from the sample chamber, the fourth electromagnetic signal generated by excitement (e.g., interrogation) of a fourth fluorophore (e.g., the Cy5.5 fluorophore) of the multiple fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590), both literally and under the doctrine of equivalents. While Cy5.5 is designated here as the example fourth fluorophore and its emission wavelength is designated as the fourth wavelength, the fourth fluorophore can be any one of the FAM, HEX, Cy5,

BIO-RAD EX.1009.031

Claims	Exemplary Disclosure of Accused Product
	Cy5.5, ROX, and ATTO 590 fluorophores that is not designated as the first, second, or third fluorophores, and the fourth wavelength is the emission wavelength of that fluorophore.
[1.c] a processor controlled analyzer to	The QX600 comprises a processor controlled analyzer, both literally and under the doctrine of equivalents. For example, operation of the QX600 Droplet Reader involves the operation of additional components (e.g., a computer, which is a processor controlled analyzer, connected to the QX600 Droplet Reader for data collection and analysis). *See, e.g.*:

BIO-RAD EX.1009.032

Claims	Exemplary Disclosure of Accused Product
	## Included Items and Additional Components This section identifies the functional items included with your droplet reader purchase, as well as additional components and consumables. For information on items you may need for a full ddPCR cycle, see Appendix E, Ordering Information. Table 5 explains the components that ship with the QX600 Droplet Reader. **Table 5. QX600 Droplet Reader and Included Components** **Component** / **Description** / **Catalog Number** QX600 Droplet Reader / Droplet reading instrument / 12013328 USB cable and power cord / USB cable that connects the computer to the instrument and power cord that connects the instrument to the power source **Note:** To obtain cables or power cords, contact Bio-Rad technical support. / Included Droplet reader plate holders (2) / Positions and secures the 96-well plate in the droplet reader plate compartment / 12006834 To ensure you have everything you need to complete runs on the QX600 Droplet Reader and analyze your results, Table 6 identifies additional components and accessories that you can purchase separately. **Table 6. Additional Accessories and Consumables** **Item** / **Description** / **Catalog Number** Computer / Computer that connects to the QX600 Droplet Reader for data collection and analysis / 12017458 96-well PCR plates / ddPCR 96-well plates / 12001925 Rainin pipets / 20 µl for sample loading 50 µl for droplet transfer 8-channel, 200 µl for oil / L-20, L8-20 L-50, L8-50 L8-200 CT0479068 at CT0479095

BIO-RAD EX.1009.033

Claims	Exemplary Disclosure of Accused Product
	The computer connected to the QX600 Droplet Reader is used to run the QX Manager Software. *See, e.g.*: **Connecting and Starting the Instrument** To execute runs on the QX600 Droplet Reader, you must connect the instrument to a computer on which the QX Manager Software is installed and configured. Bio-Rad recommends purchasing at least one configured computer that can remain connected to the QX600 Droplet Reader and be used for adding and running plates in QX Manager Software. For information on additional items you can purchase for your instrument, see Included Items and Additional Components on page 26. **To connect and start the instrument** 1. Connect the USB cable provided by Bio-Rad to the USB port on the back of the QX600 Droplet Reader, and then to the computer. 2. Press the Power button on the instrument. The Power indicator light turns solid green to indicate that power is on. 3. Turn on the connected computer and log in with a Windows account. For information, see your system administrator. 4. Open and log into QX Manager Software. **Important:** The first time you log into the software, the Instrument window appears and you must select the instrument you are using. For information on selecting the correct instrument in QX Manager Software, see Selecting the Instrument After First Sign-In on page 36. CT0479068 at CT0479097

BIO-RAD EX.1009.034

Claims	Exemplary Disclosure of Accused Product
	The computer connected to the QX600 Droplet Reader was included in sales of the QX600 or convoyed in sales of the QX600 Droplet Reader. *See, e.g.*:

BIO-RAD EX.1009.035

Claims	Exemplary Disclosure of Accused Product
	# Appendix E Ordering Information This appendix contains descriptions and catalog numbers for new or replacement instruments, accessories, and consumables for Bio-Rad's ddPCR products, including your QX600 Droplet Reader. **ddPCR System and Instrument Packages** Table 41 contains information on Bio-Rad Laboratories, Inc. Droplet Digital PCR systems, as well as individual ddPCR instruments and included accessories. **Table 41. ddPCR System and Instrument Packages** | Product	Description	Catalog Number | |---|---|---| | QX200 Droplet Digital PCR System | Includes the QX200 Droplet Generator, QX200 Droplet Reader, computer, software, and consumables | 1864001 | | QX600 Droplet Digital PCR System | Includes the QX200 Droplet Generator, QX600 Droplet Reader, computer, software, and consumables | 17007769 | | QX600 AutoDG™ Droplet Digital PCR System | Includes the Automated Droplet Generator (AutoDG), QX600 Droplet Reader, computer, software, and consumables | 17008371 | | QX200 Droplet Generator | Includes the droplet generator (1), cartridges and gaskets (24 each), cartridge holders (2), and power cord (1) | 1864002 | | QX200 Droplet Reader | Includes the droplet reader (1), plate holders (2), USB cable (1), and power cord (1) | 1864003 | | QX600 Droplet Reader | Includes the droplet reader (1), plate holders (2), USB cable (1), and power cord (1) | 12013328 | | Automated Droplet Generator | Includes the automated droplet generator (1), cooling block accessory (1), oil purge reservoir (1), and power cord (1) | 1864101 | | C1000 Thermal Cycler | Thermal cycler with 384-well reaction module | 1851384 | CT0479068 at CT0479276

BIO-RAD EX.1009.036

Claims	Exemplary Disclosure of Accused Product
	QX600 Droplet Reader Accessories Table 42 contains information on accessories for the QX600 Droplet Reader. **Table 43. QX600 Droplet Reader Accessories** Product / Description / Catalog Number Computer — Computer that connects to the QX600 Droplet Reader for data collection and analysis — 12017458 USB cable and power cord — Cable connecting the computer to the instrument and power cord connecting the instrument to the power source **Note:** Contact Bio-Rad Technical Support for replacement information. — Included Droplet reader plate holders (2) — Used to position the 96-well plate in the droplet reader plate compartment — 12006834 CT0479068 at CT0479278 Customers were also instructed to purchase a computer to the extent it was not included in their purchase of the QX600. *See e.g.* ("To ensure you have everything you need to complete runs on the QX600 Droplet reader and analyze your results, Table 6 [listing a computer] identifies additional components and accessories that you can purchase separately."):

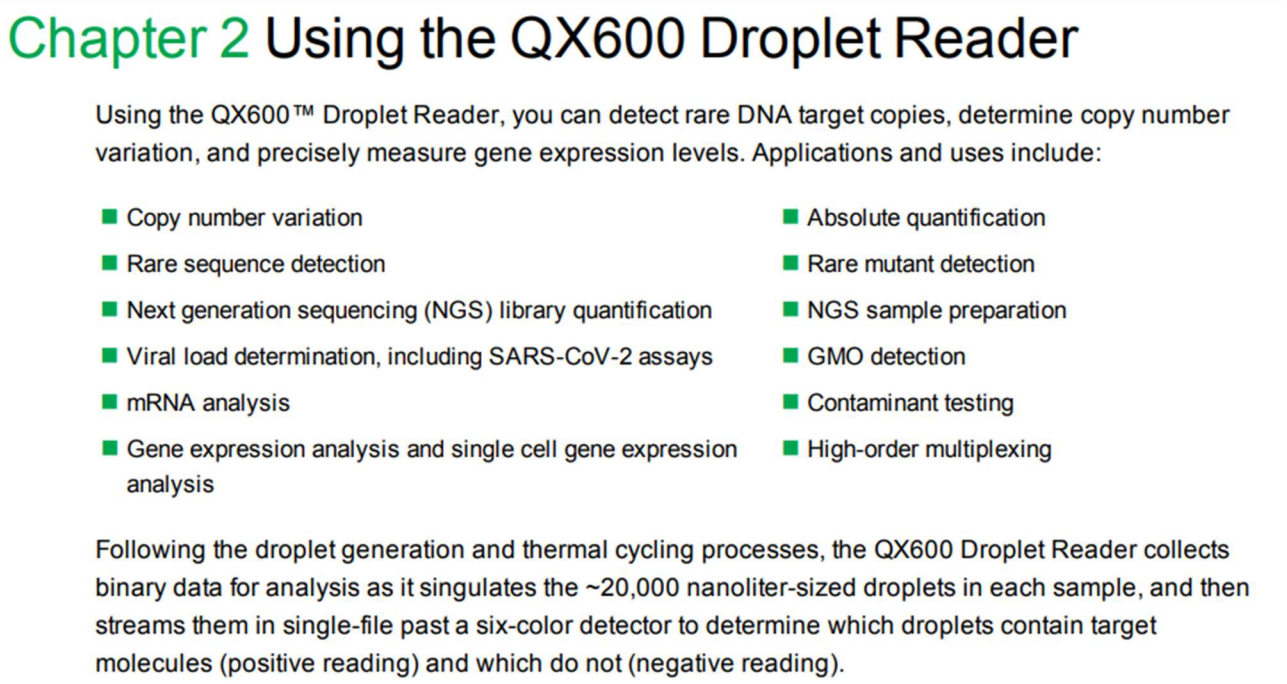
BIO-RAD EX.1009.037

Claims	Exemplary Disclosure of Accused Product
	To ensure you have everything you need to complete runs on the QX600 Droplet Reader and analyze your results, Table 6 identifies additional components and accessories that you can purchase separately. **Table 6. Additional Accessories and Consumables** **Item** / **Description** / **Catalog Number** Computer / Computer that connects to the QX600 Droplet Reader for data collection and analysis / 12017458 96-well PCR plates / ddPCR 96-well plates / 12001925 Rainin pipets / 20 µl for sample loading 50 µl for droplet transfer 8-channel, 200 µl for oil / L-20, L8-20 L-50, L8-50 L8-200 CT0479068 at CT0479095 To the extent such computer for connection to the QX600 Droplet Reader was included in sales of the QX600, or in related convoy sales, and/or because Bio-Rad induced and/or contributed to such purchase of such computer for connection to the QX600 Droplet Reader, the QX600 infringes claim 1 directly or indirectly, at least because the QX600 comprises a processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software) both literally and under the doctrine of equivalents. According to at least the examples above, the QX600 Droplet Digital PCR System comprises a processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software), both literally and under the doctrine of equivalents.

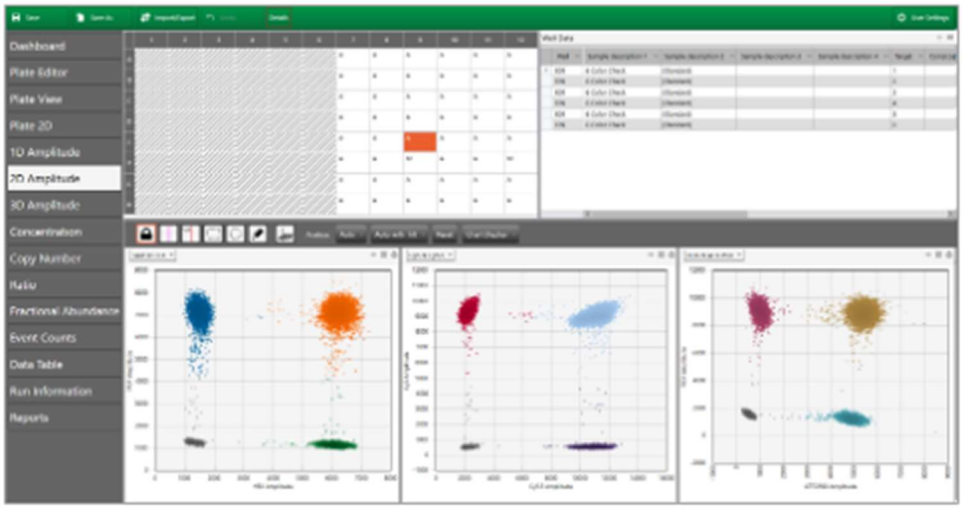
BIO-RAD EX.1009.038

Claims	Exemplary Disclosure of Accused Product
[1.c.1] receive, from the multi-channel detector, a cumulative signal based on the first, second, third, and fourth electromagnetic signals and	The processor controlled analyzer receives, from the multi-channel detector, a cumulative signal based on the first, second, third, and fourth, electromagnetic signals, both literally and under the doctrine of equivalents. For example, the QX600 Droplet Reader receives signals from respective droplets through exciting the fluorophores present in the droplets to produce electromagnetic signals and provides this data to the processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software). *See, e.g.*:

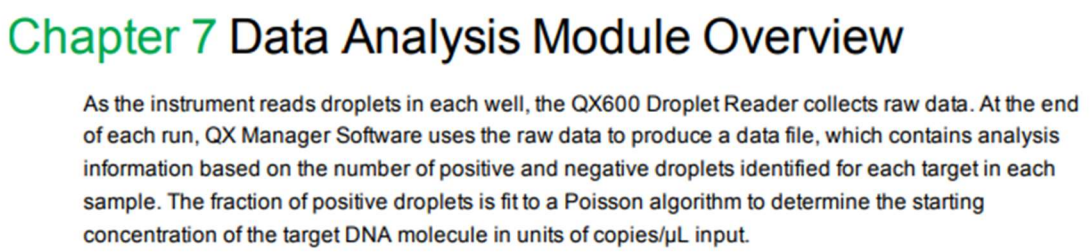
BIO-RAD EX.1009.039

Claims	Exemplary Disclosure of Accused Product
	 CT0479068 at CT0479090 The signals collect fluorescence information about the multiple fluorophores associated with target analytes within droplets, and thus the signal data is cumulative. *See, e.g.*:

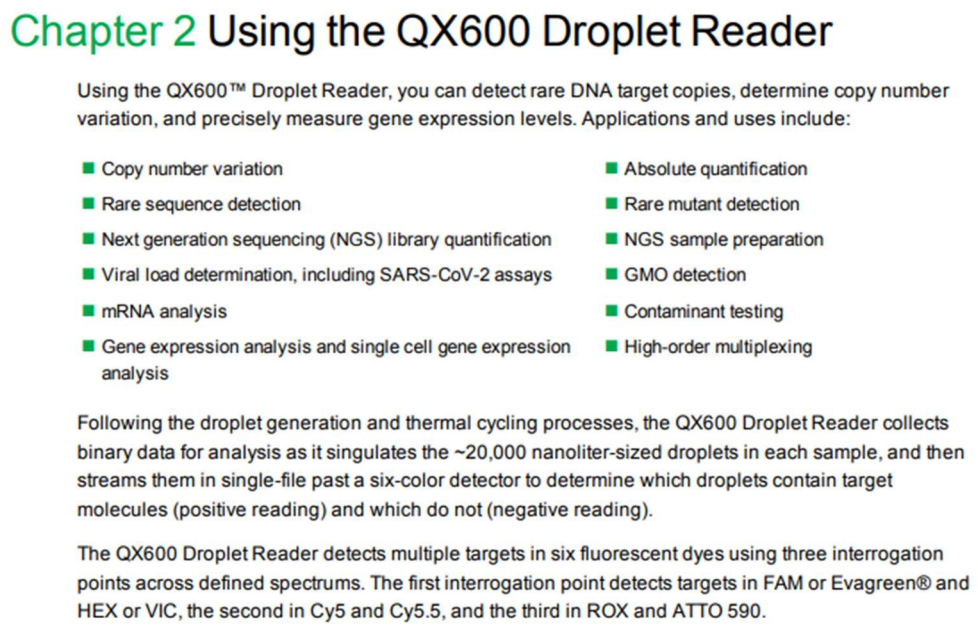
BIO-RAD EX.1009.040

Claims	Exemplary Disclosure of Accused Product
	2D Amplitude For the wells you select in the analysis view of the Plate Editor, the 2D Amplitude window illustrates fluorescence for six targets. The images in this section display data from an experiment involving six targets. Each plot shows individual cluster representations for first and second target positive droplets, droplets containing both targets, and droplets containing neither target. The following graphic shows six colors in standard-size droplets.  CT0479068 at CT0479191 The QX Manager Software receives the cumulative signal data from the QX600 Droplet Reader and produces a cumulative signal data file containing analysis information based on the number of

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Claims	Exemplary Disclosure of Accused Product
	positive and negative droplets identified for each target in the sample. *See, e.g.*:  CT0479068 at CT0479144 The cumulative signal data file comprises at least cumulative electromagnetic signal data detected by the multi-channel detector (e.g., the six-channel detector within the QX600 Droplet Reader), wherein the cumulative signal data is based on detections at a first (e.g., FAM emission wavelength), second (e.g., HEX emission wavelength), third (e.g., Cy5 emission wavelength), and fourth (e.g., Cy5.5 emission wavelength) electromagnetic signals. *See, e.g.*:

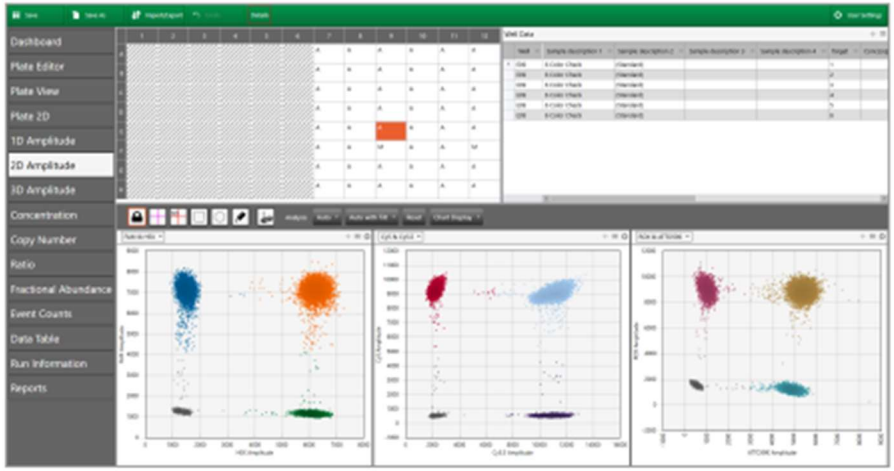
BIO-RAD EX.1009.042

Claims	Exemplary Disclosure of Accused Product
	 Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 According to at least the examples above, the processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software) receives, from the multi-channel detector (e.g., the six-channel detector within the QX600 Droplet Reader), a cumulative signal based on the first (e.g., FAM emission wavelength), second (e.g., HEX emission wavelength), third (e.g., Cy5 emission wavelength), and fourth (e.g., Cy5.5 emission wavelength) electromagnetic signals, both literally and under the doctrine of equivalents.

BIO-RAD EX.1009.043

Claims	Exemplary Disclosure of Accused Product
[1.c.2.A] apply a decoding matrix to the cumulative signal to unambiguously detect the presence or absence of at least each of M analytes	The processor controlled analyzer applies a decoding matrix to the cumulative signal to unambiguously detect the presence or absence of at least each of M analytes, both literally and under the doctrine of equivalents. For example, the processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software) presents analysis data in different formats, including chromatograms, wherein threshold lines are used to unambiguously distinguish positive or negative droplet counts as indications of the presence or absence of analytes. *See, e.g.*: Chapter 8 Data Analysis Methodology As summarized in Chapter 7, Data Analysis Module Overview, the software presents analysis data in several different formats. When you open the data file in the Analysis module, you can view the data with the automatic analysis thresholds and calculations or you can choose different options to recalculate, replot, or recolor the results. Data is shown in the following formats: ■ Amplitude, which shows each droplet in each well in a scatter plot, in views ■ Data visualizations, which show estimated target molecule concentration, ratio, and fractional abundance of one target against another target based on selected error model and confidence interval **Note:** Copy number variation is also calculated if the user ran a CNV experiment. ■ Event counts, which are precise counts of positive and negative droplets for each target in each well

BIO-RAD EX.1009.044

Claims	Exemplary Disclosure of Accused Product
	CT0479068 at CT0479180 **2D Amplitude** For the wells you select in the analysis view of the Plate Editor, the 2D Amplitude window illustrates fluorescence for six targets. The images in this section display data from an experiment involving six targets. Each plot shows individual cluster representations for first and second target positive droplets, droplets containing both targets, and droplets containing neither target. The following graphic shows six colors in standard-size droplets.  CT0479068 at CT0479191 The processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running

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Claims	Exemplary Disclosure of Accused Product
	the QX Manager Software) utilizes thresholds. *See, e.g.*:

Note: Well data must meet certain quality metrics (minimum of 10,000 droplets) before QX Manager Software will automatically calculate a threshold above which droplets are considered positive. You can use the manual thresholding tools to ensure correct quantification of positive and negative droplets.

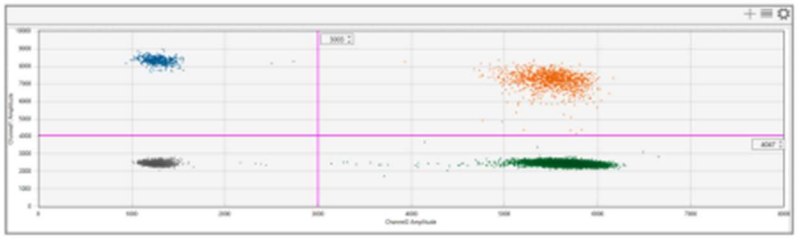
To use the 2D amplitude tools

1. Open your data file in the Analysis module and select the 2D Amplitude tab.
2. Choose a threshold line or cluster option.

   Note: Use the cluster option when the data is not orthogonal and the threshold lines do not adequately divide the populations of droplets.

   - To adjust threshold lines, select a threshold line type (

) and then click in the plot to apply crosshair lines. Use any of the following methods:
     - Drag the lines horizontally or vertically in the plot area.
     - Enter a new threshold value in the boxes at the end of each threshold line.
     - Tap the up and down arrows to incrementally adjust the numbers.



BIO-RAD EX.1009.046

Claims	Exemplary Disclosure of Accused Product
	CT0479068 at CT0479192 The processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software) recognizes certain assay types, including Amplitude Multiplex assay types. *See, e.g.*: Appendix A Experiment Examples This section contains setup and analysis information for the following sample experiments: ■ Direct Quantification, using the Amplitude Multiplex assay type ■ Copy Number Variation, using the Probe Mix Triplex assay type ■ Drop-Off, using the Basic Drop-off Assay type for genome edit detection ■ Direct Quantification, using the Advanced Classification Method assay type CT0479068 at CT0479250 When analyzing Amplitude Multiplex assay types, the processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software) uses the thresholds to set the boundaries of discrete signal intensity levels discrete channel intensity levels (*e.g.*, None, Hi, Lo). The processor controlled analyzer assigns up to M=12 targets to the discrete channel intensity levels across the QX600's six channels. *See, e.g.*:

BIO-RAD EX.1009.047

Claims	Exemplary Disclosure of Accused Product

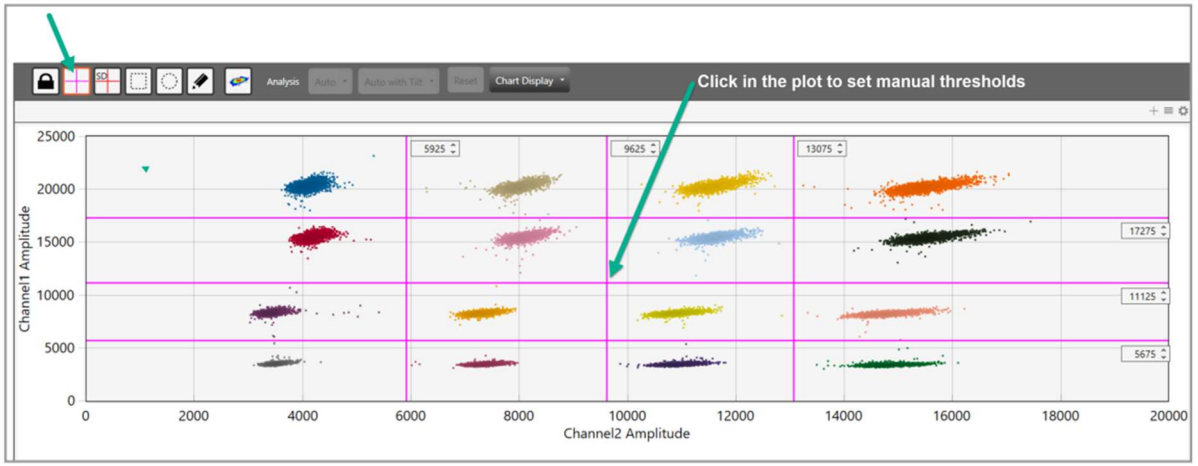
Table 17. Fluorophore options

Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4	Signal Ch5	Signal Ch6
Single target per channel, for1 to 6 targets	FAM EvaGreen® None	HEX VIC None	Cy5 None	Cy5.5 None	ROX None	ATTIO590 None
Amplitude multiplex, for 1 to 12 targets	FAM Hi FAM Lo EvaGreen® Hi EvaGreen® Lo None	HEX Hi HEX Lo VIC Hi VIC Lo None	Cy5 Hi Cy 5 Lo None	Cy5.5 Hi Cy 5.5 Lo None	ROX Hi ROX Lo None	ATTO 590 Hi ATTO 590 Lo None
Probe mix triplex, for 9 targets	FAM None	HEX VIC None	Cy5 None	Cy5.5 None	ROX None	ATTO 590 None
Advanced classification	FAM +1-10 EvaGreen® 1 to 10 None	HEX 1 to 10 VIC 1 to 10 None	Cy5 1 to 10 None	Cy5.5: 1 to 10 None	ROX 1 to 10 None	ATTO 590 1 to 10 None

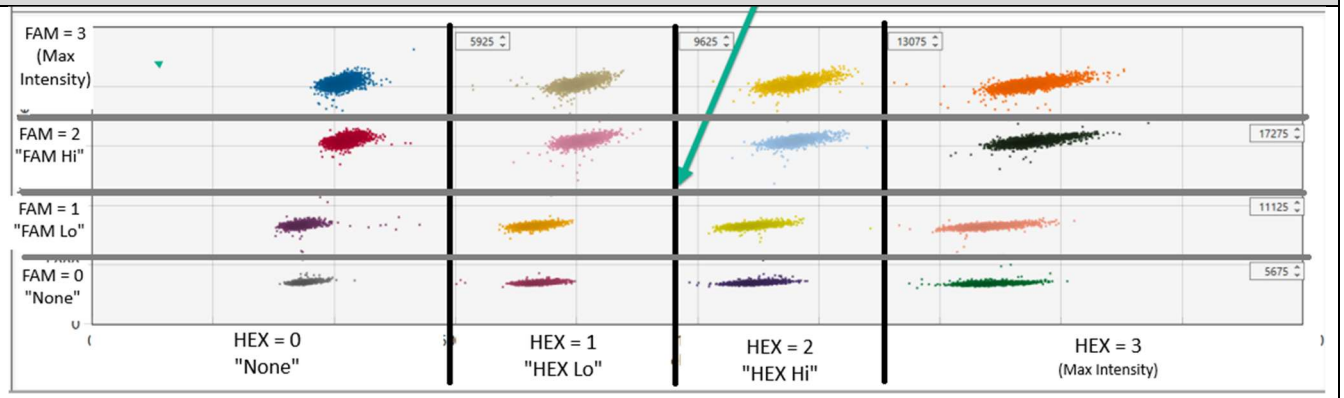
CT0479068 at CT0479141

The boundaries of these discrete channel intensity levels are set by thresholds. *See, e.g.*:

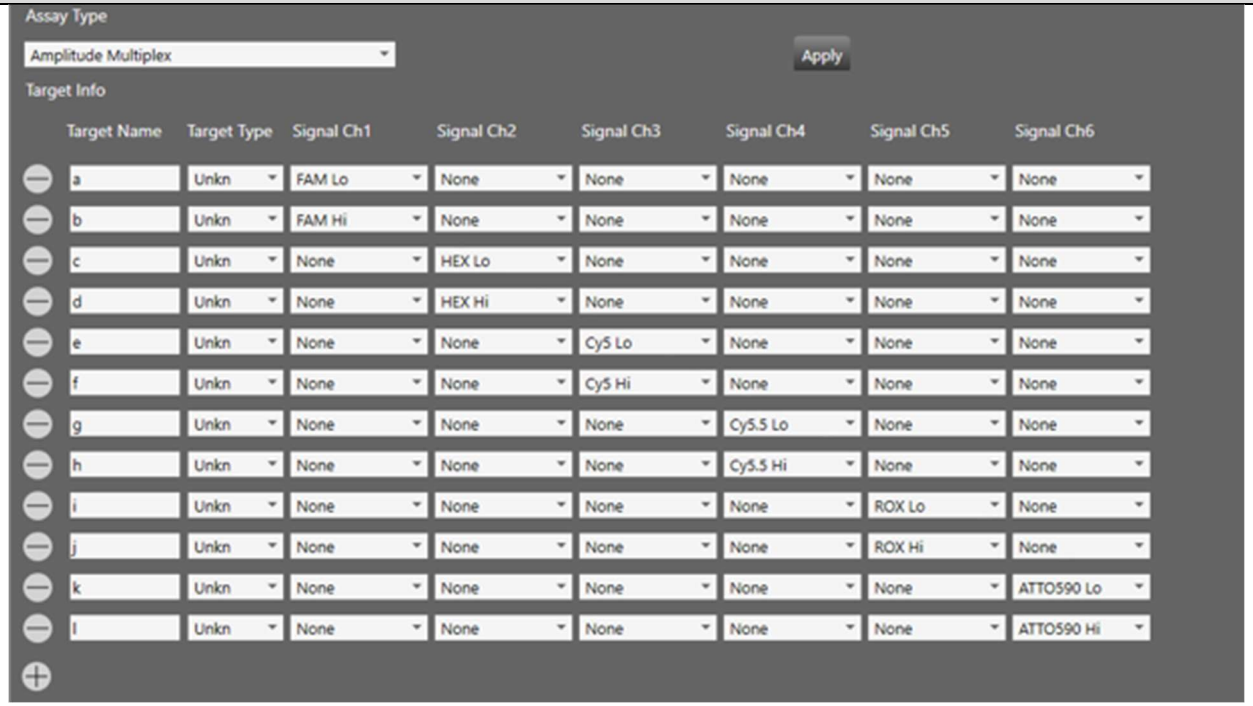
BIO-RAD EX.1009.048

Claims	Exemplary Disclosure of Accused Product
	 CT0479068 at CT0479252 The thresholds above thus are understood to discretize the signal intensity values measured on each of the two channels on each plot into four levels 0, 1, 2, and 3:

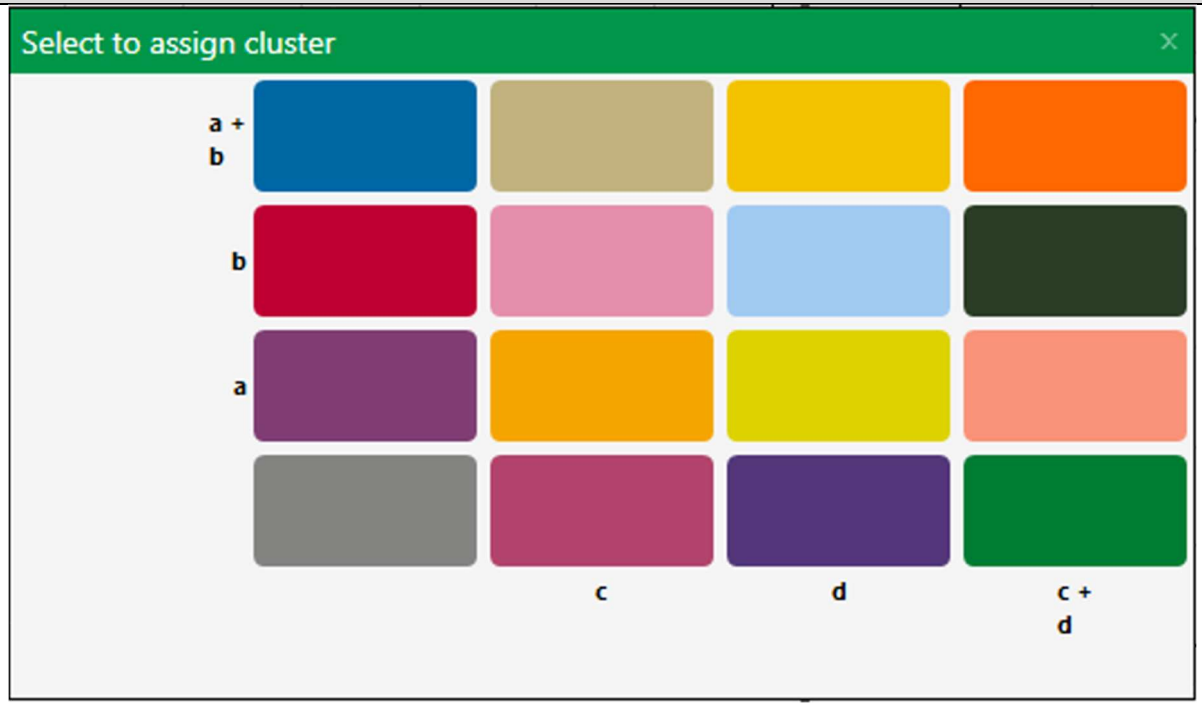
BIO-RAD EX.1009.049

Claims	Exemplary Disclosure of Accused Product
	 CT0479068 at CT0479252 (notation of discretized channel signal intensity levels added, threshold line emphasis added) Upon naming of the target analytes in the QX Manager Software, the processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software) automatically assigns positive droplet readings within specific regions of the chromatogram to specific combinations of the named target analytes. For example, selecting Amplitude Multiplexing assay type and naming twelve targets a, b, c, d, e, f, g, h, i, j, k, and l:

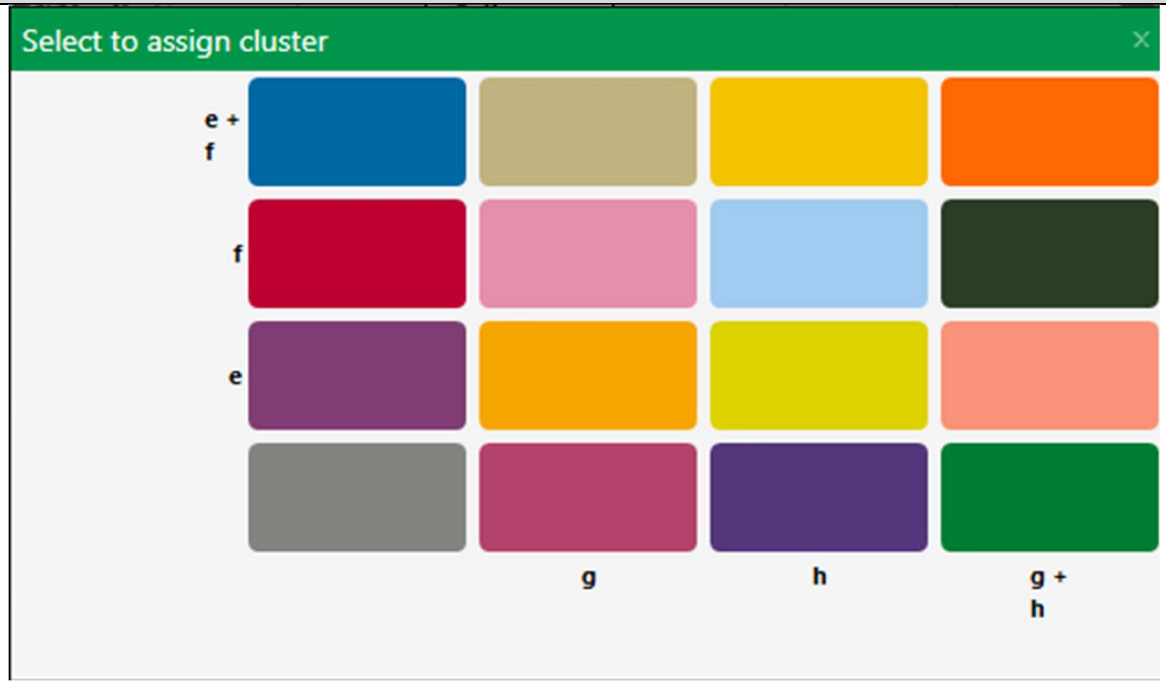
BIO-RAD EX.1009.050

Claims	Exemplary Disclosure of Accused Product
	 CT0478185 When a user attempts to set thresholds on the 2D chromatograms, the QX Manager Software interface reveals the default assignment matrix for amplitude multiplexing assay types. *See e.g.*: For channels 1 and 2 (FAM and HEX):

BIO-RAD EX.1009.051

Claims	Exemplary Disclosure of Accused Product
	 CT0478184 For channels 3 and 4 (Cy5 and Cy5.5):

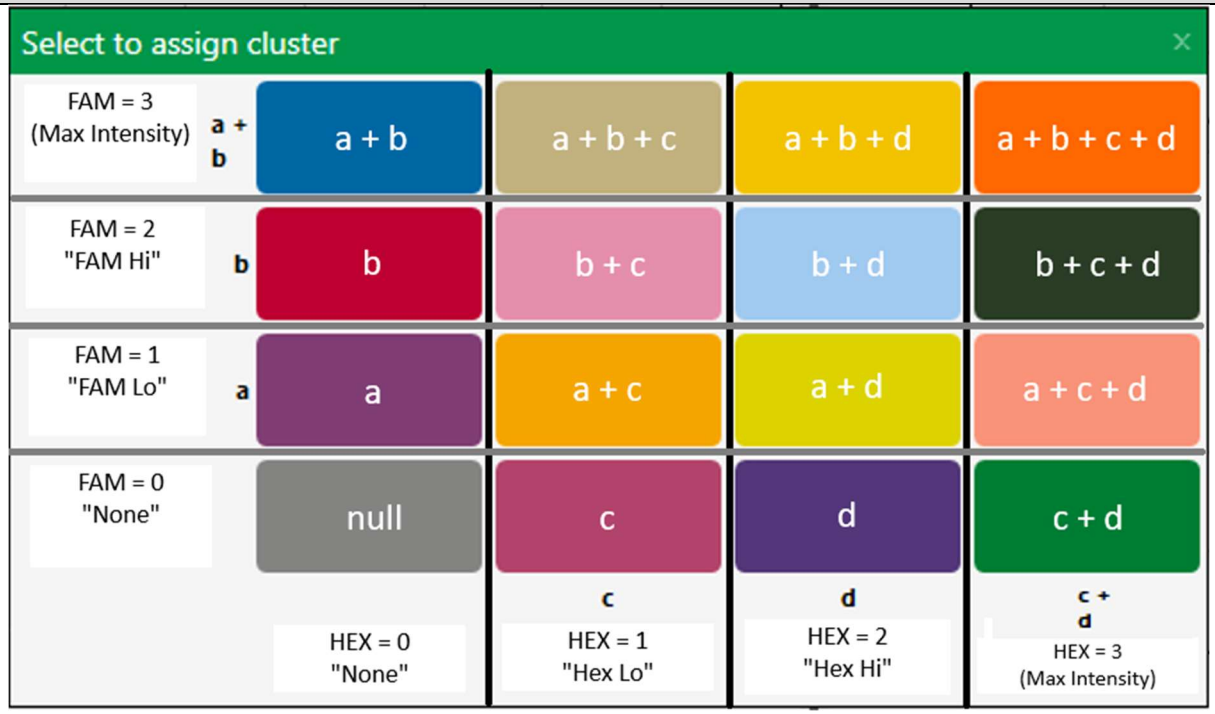
BIO-RAD EX.1009.052

Claims	Exemplary Disclosure of Accused Product
	 CT0478186 For channels 5 and 6 (ROX and ATTO 590):

BIO-RAD EX.1009.053

Claims	Exemplary Disclosure of Accused Product
	 CT0478187 Overlaying the discretized signal intensity levels described above in relation to the threshold lines shows how the specific regions relate to the detected signal intensity levels:

BIO-RAD EX.1009.054

Claims	Exemplary Disclosure of Accused Product
	 CT0478184 (notation of discretized channel signal intensity levels added, threshold line emphasis added, white text indicating each assigned specific combination of analytes added) On information and belief, the processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software) applies a decoding matrix to assign the above target analyte combinations to the available regions formed by the discretized signal channel levels, and then droplet data with signal channel levels falling within a given discretized signal channel region is associated with the target analyte combination assigned to that region.

BIO-RAD EX.1009.055

<table>
<tr><th>Claims</th><th>Exemplary Disclosure of Accused Product</th></tr>
<tr><td></td><td>
Alternatively, or in addition, the processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software) uses a function substantially the same as applying a decoding matrix to the cumulative signal (e.g., assigning the droplet data to target analyte combinations according to how fluorophore intensity levels in the droplet data relate to defined thresholds), in the same way of using cumulative signal data based on the first, second, third, and fourth electromagnetic signals to achieve the same result of unambiguously detecting the presence or absence of at least each of M analytes.

The decoding matrix for channels 1 and 2 may be represented as the following:

<table>
<tr><th>Analyte</th><th>FAM</th><th>HEX</th></tr>
<tr><td>a</td><td>1</td><td>0</td></tr>
<tr><td>b</td><td>2</td><td>0</td></tr>
<tr><td>c</td><td>0</td><td>1</td></tr>
<tr><td>d</td><td>0</td><td>2</td></tr>
</table>

Expanding this same analysis to the default target combination assignments to all six channels as provided by the QX Manager interface, the following larger decoding table covering all twelve target analytes may be derived:

<table>
<tr><th>Analyte</th><th>FAM</th><th>HEX</th><th>Cy5</th><th>Cy5.5</th><th>ROX</th><th>ATTO 590</th></tr>
<tr><td>a</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>b</td><td>2</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>c</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>d</td><td>0</td><td>2</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>e</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>f</td><td>0</td><td>0</td><td>2</td><td>0</td><td>0</td><td>0</td></tr>
</table>
</td></tr>
</table>

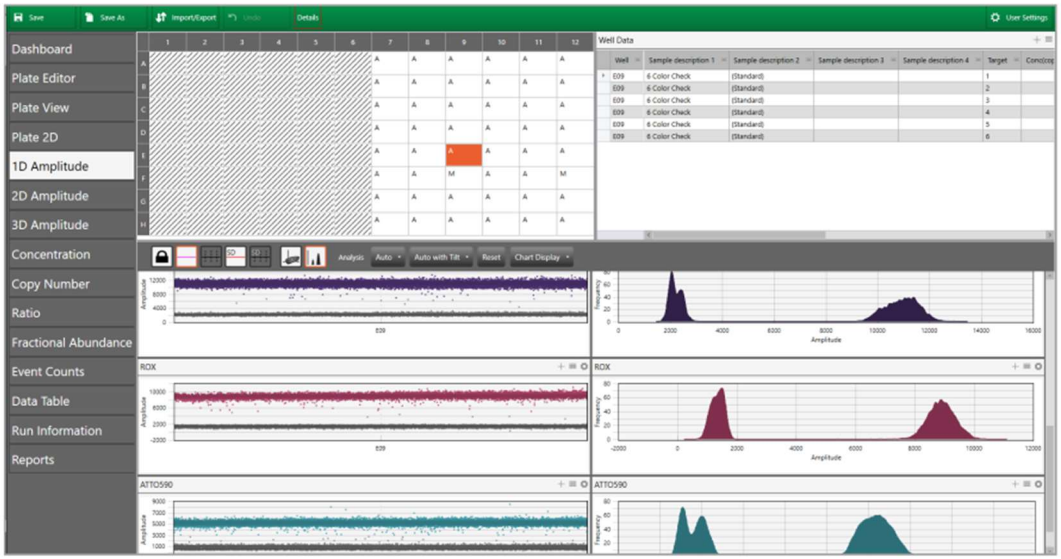
BIO-RAD EX.1009.056

Claims	Exemplary Disclosure of Accused Product
	<table><tr><td>**g**</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td></tr><tr><td>**h**</td><td>0</td><td>0</td><td>0</td><td>2</td><td>0</td><td>0</td></tr><tr><td>**i**</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td></tr><tr><td>**j**</td><td>0</td><td>0</td><td>0</td><td>0</td><td>2</td><td>0</td></tr><tr><td>**k**</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td></tr><tr><td>**l**</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>2</td></tr></table> According to at least the examples above, the processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software) applies a decoding matrix to the cumulative signal (e.g. assigns target analyte combinations to discrete signal channel levels and associates droplet data with target analyte combinations according to signal channel levels within the droplet data) to unambiguously detect the presence or absence of at least each of M analytes, both literally and under the doctrine of equivalents.
[1.c.2.B] by associating, for each analyte, a first value in a first component of the cumulative signal and a second value in a second component of the cumulative signal, wherein each first value is an intensity or range of intensities and each	The processor controlled analyzer applies the decoding matrix to the cumulative signal data by associating for each analyte, a first value in a first component of the cumulative signal and a second value in a second component of the cumulative signal, wherein each first value is an intensity or range of intensities and each second value is a wavelength or a range of wavelengths, and wherein the second values comprise the first, second, third, and fourth wavelengths, both literally and under the doctrine of equivalents. For example, as explained in [1.c.2.A] above, the processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software) associates signal intensity levels with certain combinations of analytes. The QX600 Droplet Reader collects intensity measurements from three interrogation points covering

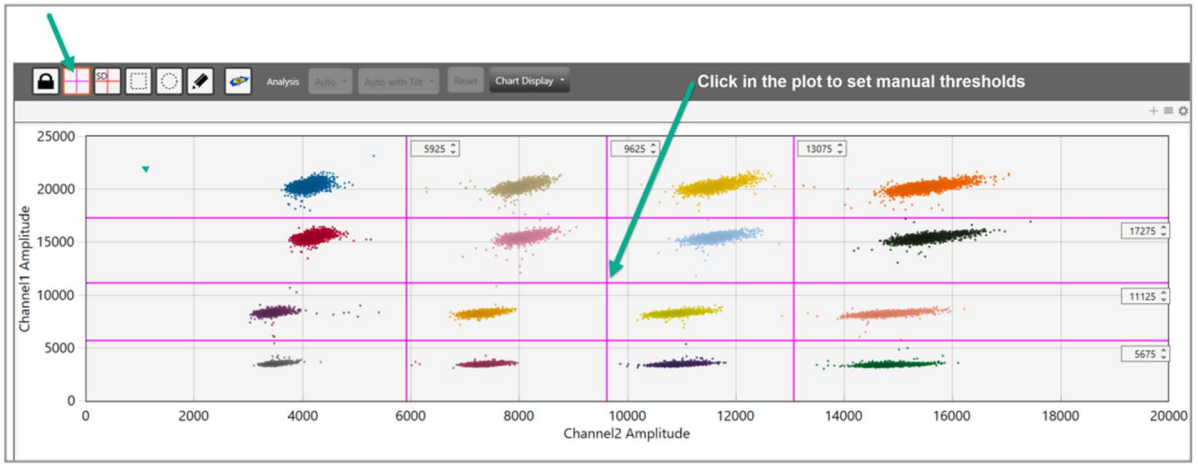
BIO-RAD EX.1009.057

Claims	Exemplary Disclosure of Accused Product
second value is a wavelength or a range of wavelengths, and wherein the second values comprise the first, second, third, and fourth wavelengths, and	six fluorophore wavelength channels (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590). *See. e.g.*: Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 The QX600 Droplet Reader cumulative signal includes signal intensity (e.g., amplitude) values for each fluorophore wavelength. *See e.g.*:

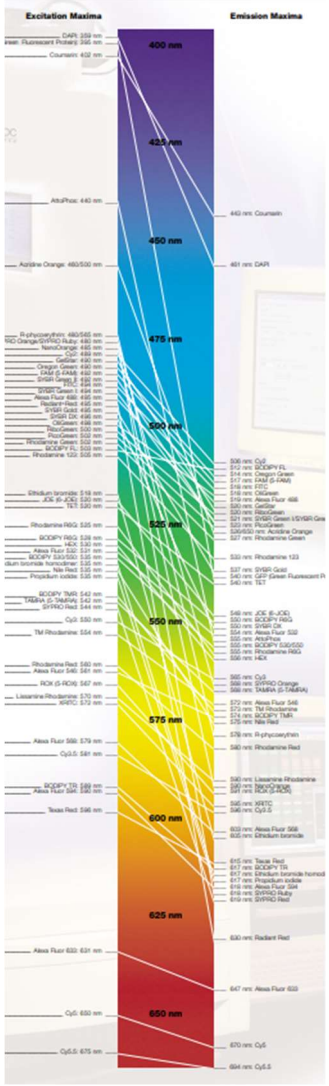
BIO-RAD EX.1009.058

Claims	Exemplary Disclosure of Accused Product
	1D Amplitude Use the 1D Amplitude analysis window to view droplet amplitudes for each channel, in selection order, for each well selected in the Well Selector. The following graphic shows six colors in standard-size droplets.  CT0479068 at CT0479186

BIO-RAD EX.1009.059

Claims	Exemplary Disclosure of Accused Product
	 CT0479068 at CT0479252 Each of the fluorophores has a specific emission wavelength or a range of wavelengths. The emission wavelength for FAM is 517 nm, the emission wavelength for HEX is 556 nm, the emission wavelength for Cy5 is 670 nm, the emission wavelength for Cy5.5 is 694 nm, the emission wavelength for ROX is 591 nm, and the emission wavelength for ATTO 590 is 622 nm. *See, e.g.*:

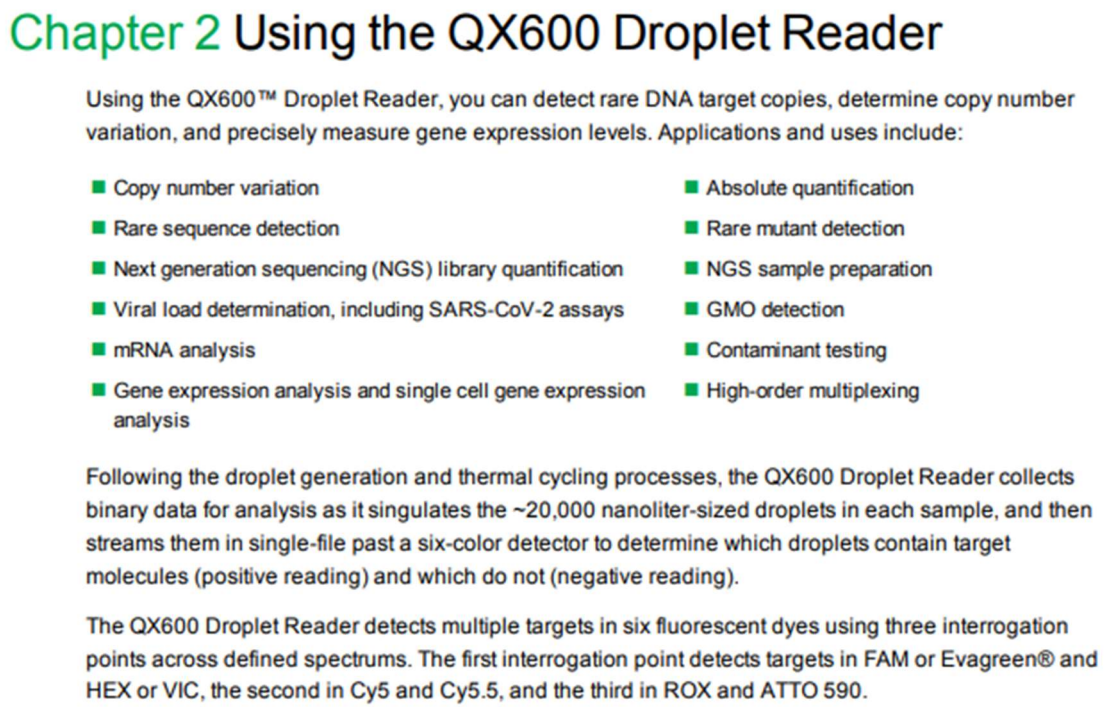
BIO-RAD EX.1009.060

Claims	Exemplary Disclosure of Accused Product
	

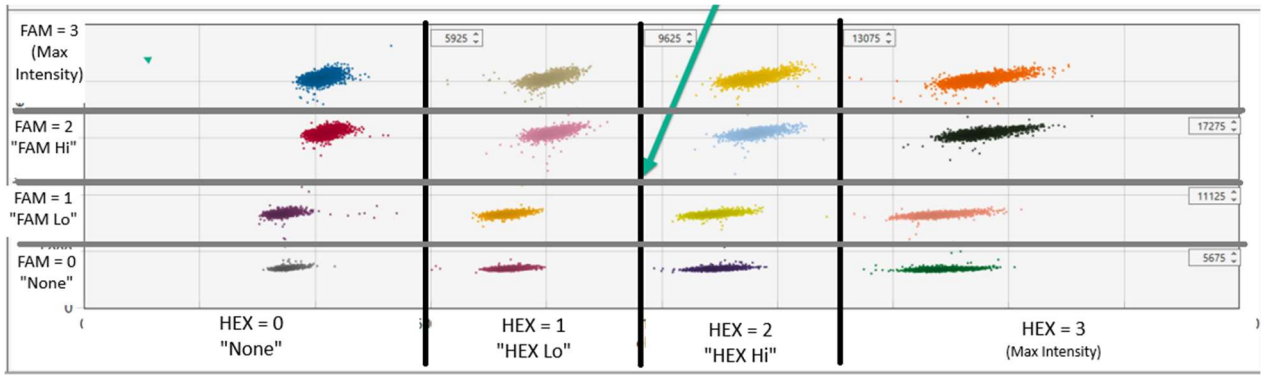
BIO-RAD EX.1009.061

Claims	Exemplary Disclosure of Accused Product
	CT0479364 at CT0479364 According to at least the above examples, the processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software) applies the decoding matrix (e.g. assigns target analyte combinations to discrete signal channel levels and associates droplet data with target analyte combinations according to signal channel levels within the droplet data) to the cumulative signal data by associating for each analyte, a first value (e.g., a FAM fluorescence intensity value), in a first component of the cumulative signal and a second value (e.g., a FAM emission wavelength value), in a second component of the cumulative signal, wherein each first value (e.g. a FAM fluorescence intensity value, a HEX fluorescence intensity value, a Cy5 fluorescence intensity value, a Cy5.5 fluorescence intensity value, a ROX fluorescence intensity value, an ATTO 590 fluorescence intensity value), is an intensity or range of intensities and each second value (e.g., a FAM emission wavelength value, a HEX emission wavelength value, a Cy5 emission wavelength value, a Cy5.5 emission wavelength value, a ROX emission wavelength value, an ATTO 590 emission wavelength value) is a wavelength or a range of wavelengths, and wherein the second values comprise the first (e.g., FAM wavelength, or 517 nm), second (e.g., HEX wavelength, or 556 nm), third (e.g., Cy5 wavelength, or 670 nm) and fourth (e.g., Cy5.5 wavelength, or 694 nm) wavelengths, both literally and under the doctrine of equivalents.
[1.c.2.C] the determination is made without immobilization, mass spectrometry or melting curve analysis;	Caltech incorporates the discussion regarding above elements of claim 1, *supra*, in their entirety. As discussed in relation to those elements, the unambiguous determination of the presence or absence of at least each of the M analytes in the sample is made without immobilization, mass spectrometry, or melting curve analysis.
[1.c.2.D] wherein for the positive	The processor controlled analyzer makes determinations wherein, for the positive integer M, M=C*log2(F+1), both literally and under the doctrine of equivalents.

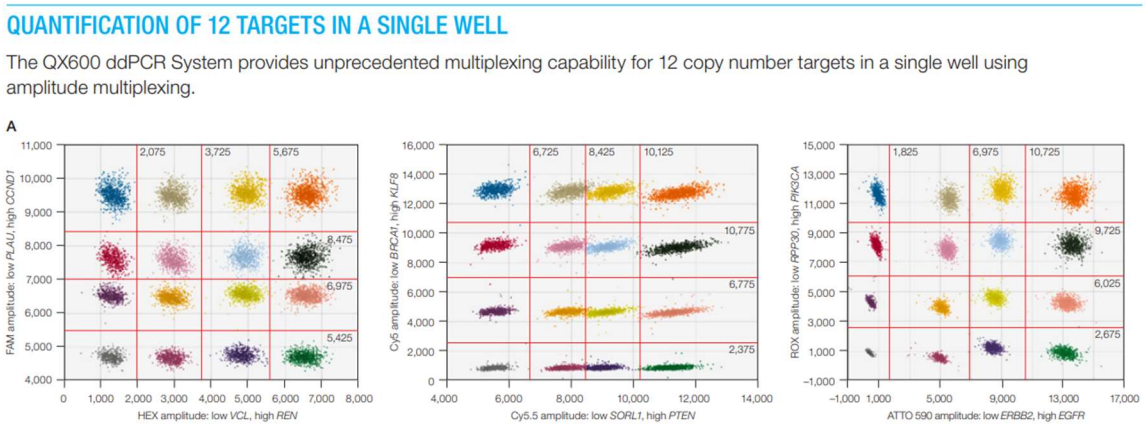
BIO-RAD EX.1009.062

Claims	Exemplary Disclosure of Accused Product
integer M, M=C*log2(F+1),	For example, where intensity data is collected from all six channels the number of second values, C (e.g., fluorophore wavelengths) is six. *See. e.g.*:  CT0479068 at CT0479090 The processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software) applies a decoding matrix to the cumulative signal (e.g. assigns target analyte combinations to discrete signal channel levels and associates droplet data with target analyte

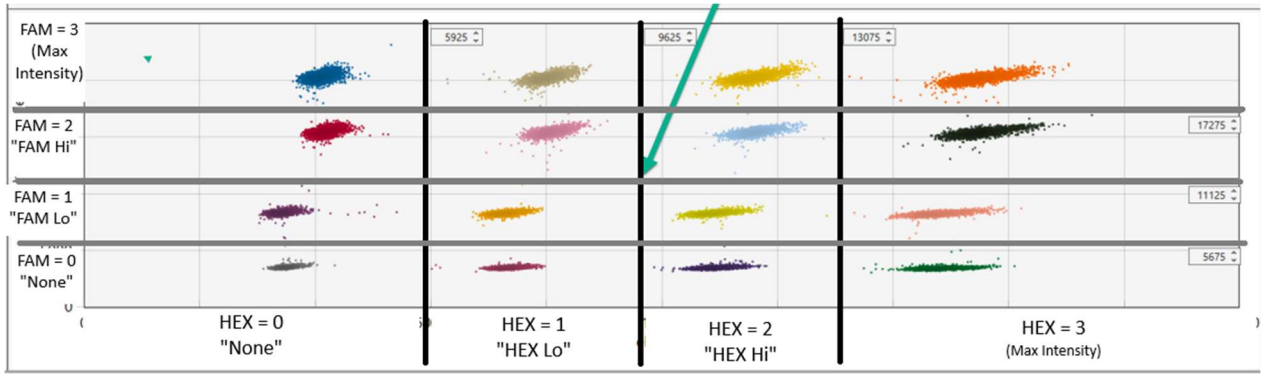
BIO-RAD EX.1009.063

Claims	Exemplary Disclosure of Accused Product
	combinations according to signal channel levels within the droplet data), such that maximum cumulative intensity for any channel, F, is three. *See, e.g.*:  CT0479068 at CT0479252 (notation of discretized channel signal intensity levels added, threshold line emphasis added) Plugging C=6 and F=3 into the equation M=C*log2(F+1), calculates an analyte count of twelve: M=C*log2(F+1) M=6*log2(3+1) M=6*log2(4) M=6*2 M=12

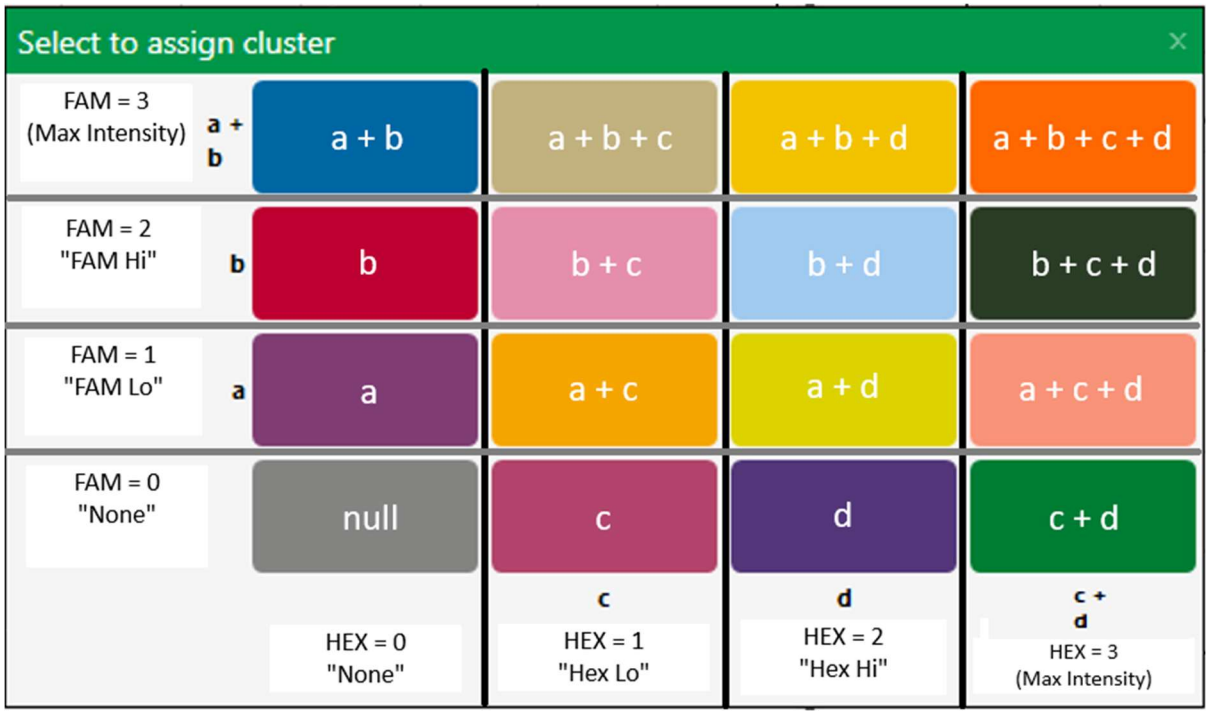
BIO-RAD EX.1009.064

Claims	Exemplary Disclosure of Accused Product
	This is consistent with the number of analytes unambiguously determined to be present or absent when the QX600 uses six channels, which is twelve analytes. *See e.g.* (twelve analytes unambiguously detected are PLAU, CCND1, VCL, REN, BRCA1, KLF8, SORL1, PTEN, RPP30, PIK3CA, ERBB2, and EGFR):  CT0479366 at CT0479368 According at least to the above examples, the processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software) makes determinations wherein, for the positive integer M (e.g., 12), M=C*log2(F+1) (e.g., 12=6*log2(3+1)), both literally and under the doctrine of equivalents.

BIO-RAD EX.1009.065

Claims	Exemplary Disclosure of Accused Product
[1.c.2.E] F is a positive integer and is equal to the maximum cumulative intensity of the first component of the signal, for any second value, when all of the analytes are present, and	The processor controlled analyzer makes determinations wherein F is a positive integer and is equal to the maximum cumulative intensity of the first component of the signal, for any second value, when all of the analytes are present, both literally and under the doctrine of equivalents. For example, the processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software) applies a decoding matrix to the cumulative signal (e.g. assigns target analyte combinations to discrete signal channel levels and associates droplet data with target analyte combinations according to signal channel levels within the droplet data), such that maximum cumulative intensity for any channel, F, is three. *See, e.g.*:  CT0479068 at CT0479252 (notation of discretized channel signal intensity levels added, threshold line emphasis added) F=3 and three is a positive integer. Where signal intensity on both the FAM and HEX channels exceeds the threshold for a discrete valuation of three, the detection is classified in the upper right orange region, indicating the presence

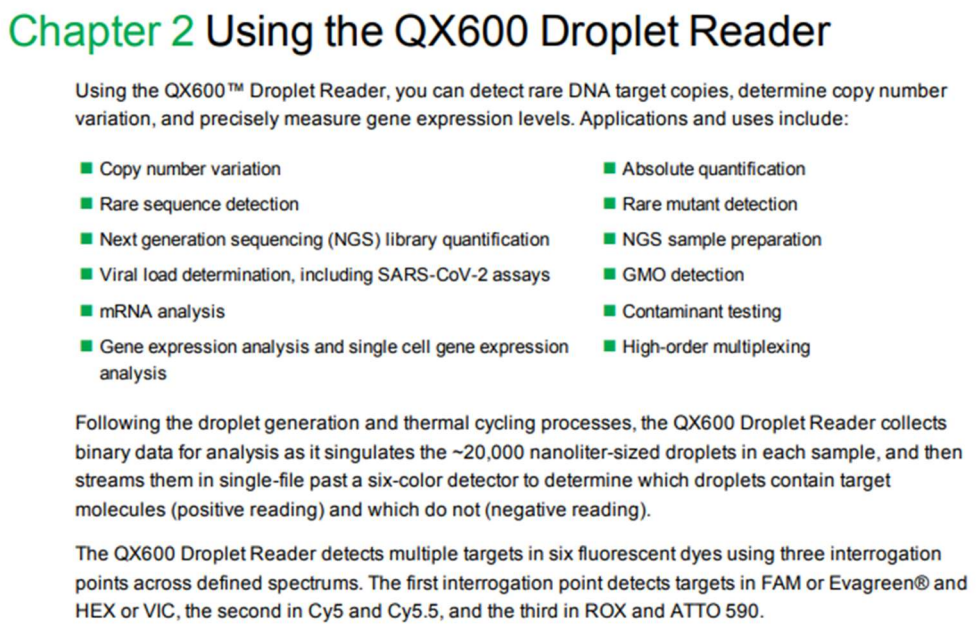
BIO-RAD EX.1009.066

Claims	Exemplary Disclosure of Accused Product
	of all analytes (a + b + c + d). *See, e.g.*:  CT0478184 (notation of discretized channel signal intensity levels added, threshold line emphasis added, white text indicating each assigned specific combination of analytes added) The orange cluster represents droplets containing all of the analytes of the chromatogram. If signal intensity on all channels (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590) exceeds the threshold for a discrete valuation of three, then the sample would be classified as containing all analytes. According at least to the above examples, the processor controlled analyzer (e.g., a computer

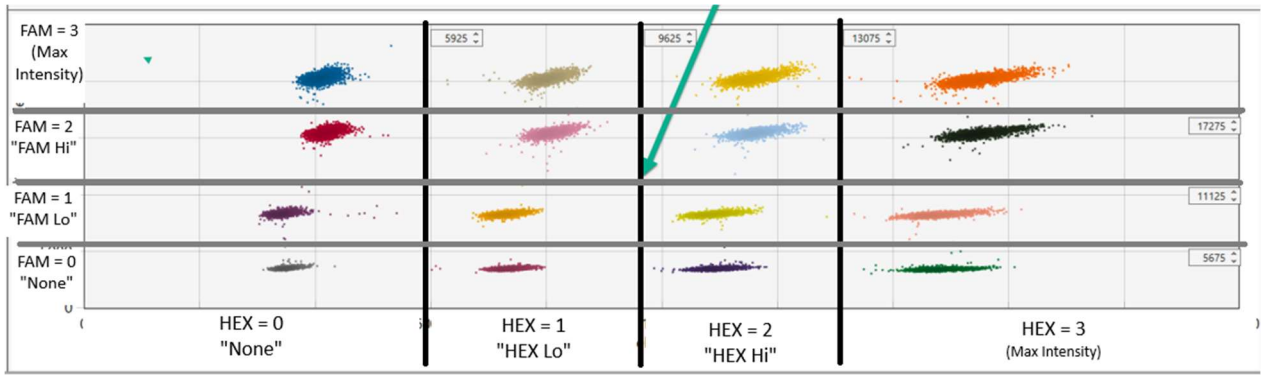
BIO-RAD EX.1009.067

Claims	Exemplary Disclosure of Accused Product
	connected to the QX600 Droplet Reader running the QX Manager Software) makes determinations wherein F is a positive integer (e.g., 3) and is equal to the maximum cumulative intensity of the first component of the signal, for any second value (e.g., intensity at FAM, HEX, Cy5, Cy5.5, ROX, or ATTO 590 emission wavelengths) when all of the analytes are present, both literally and under the doctrine of equivalents.
[1.c.2.F] C=4, 5, or 6; and	The processor controlled analyzer makes determinations wherein C=4, 5, or 6, both literally and under the doctrine of equivalents. For example, the QX600 Droplet Reader collects intensity data from up to six channels (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590), so the number of second values, C (e.g., fluorophore wavelengths) may be 1, 2, 3, 4, 5, or 6. *See, e.g.*:

BIO-RAD EX.1009.068

Claims	Exemplary Disclosure of Accused Product
	 Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 According at least to the above examples, the processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software) makes determinations wherein C=4, 5, or 6 (e.g., 6), both literally and under the doctrine of equivalents.

BIO-RAD EX.1009.069

Claims	Exemplary Disclosure of Accused Product
[1.c.2.G] wherein F+1 is a positive integer and wherein F+1 is a power of 2,	The processor controlled analyzer makes determinations wherein F+1 is a positive integer and wherein F+1 is a power of 2, both literally and under the doctrine of equivalents. For example, The processor controlled analyzer applies a decoding matrix to the cumulative signal (e.g. assigns target analyte combinations to discrete signal channel levels and associates droplet data with target analyte combinations according to signal channel levels within the droplet data), such that maximum cumulative intensity for any channel, F, is three. *See, e.g.*:  CT0479068 at CT0479252 (notation of discretized channel signal intensity levels added, threshold line emphasis added) F=3 in the above examples, and F+1=4. Four is a positive integer and a power of two, e.g. 2^2=4. According at least to the above examples, the processor controlled analyzer (e.g., a computer

BIO-RAD EX.1009.070

Claims	Exemplary Disclosure of Accused Product
	connected to the QX600 Droplet Reader running the QX Manager Software) makes determinations wherein F+1 is a positive integer (e.g., 4) and wherein F+1 is a power of 2 (e.g., 4=2^2), both literally and under the doctrine of equivalents.
[1.c.2.H] wherein M is greater than the number of the second values used to encode the analytes (C), the multi-channel detector comprises C channels, and M and C are positive integers.	The processor controlled analyzer makes determinations wherein M is greater than the number of the second values used to encode the analytes (C), the multi-channel detector comprises C channels, and M and C are positive integers, both literally and under the doctrine of equivalents. For example, the QX600 Droplet Reader detects analytes using up to six color channels (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590) to detect up to twelve targets. *See. e.g.*:

BIO-RAD EX.1009.071

Claims	Exemplary Disclosure of Accused Product
	# Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090

BIO-RAD EX.1009.072

Claims	Exemplary Disclosure of Accused Product

Table 17. Fluorophore options

Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4	Signal Ch5	Signal Ch6
Single target per channel, for1 to 6 targets	FAM EvaGreen® None	HEX VIC None	Cy5 None	Cy5.5 None	ROX None	ATTIO590 None
Amplitude multiplex, for 1 to 12 targets	FAM Hi FAM Lo EvaGreen® Hi EvaGreen® Lo None	HEX Hi HEX Lo VIC Hi VIC Lo None	Cy5 Hi Cy 5 Lo None	Cy5.5 Hi Cy 5.5 Lo None	ROX Hi ROX Lo None	ATTO 590 Hi ATTO 590 Lo None
Probe mix triplex, for 9 targets	FAM None	HEX VIC None	Cy5 None	Cy5.5 None	ROX None	ATTO 590 None
Advanced classification	FAM +1-10 EvaGreen® 1 to 10 None	HEX 1 to 10 VIC 1 to 10 None	Cy5 1 to 10 None	Cy5.5: 1 to 10 None	ROX 1 to 10 None	ATTO 590 1 to 10 None

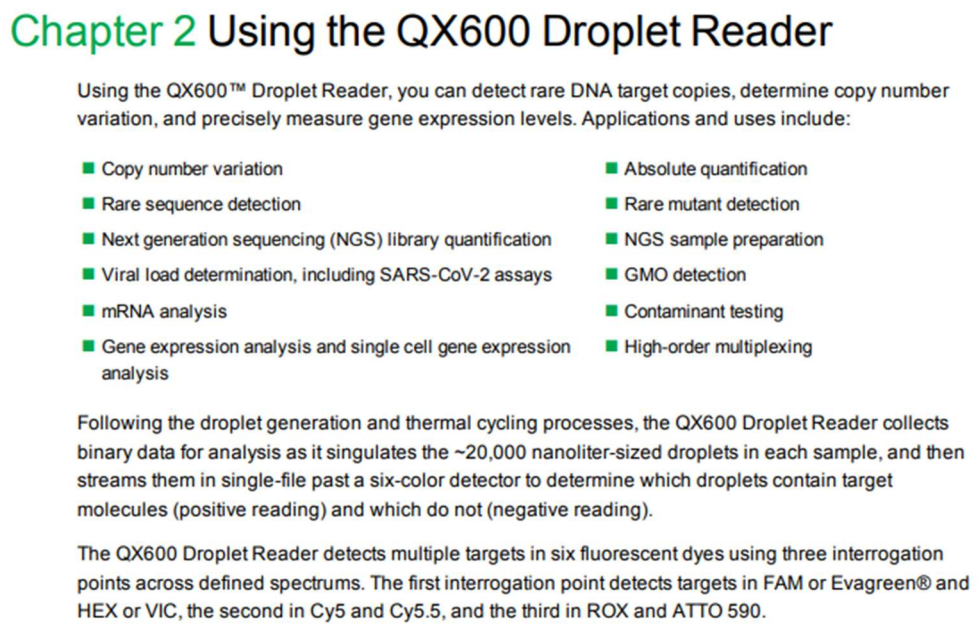
CT0479068 at CT0479141

Where intensity data is collected from six colors, the number of second values, C (e.g., fluorophore

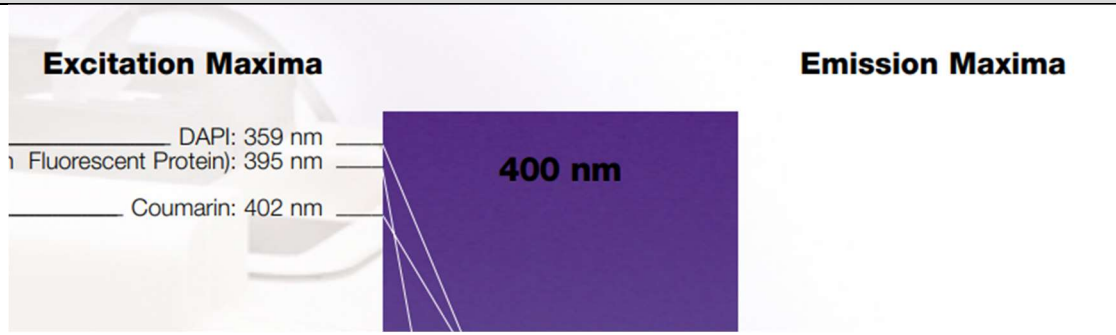
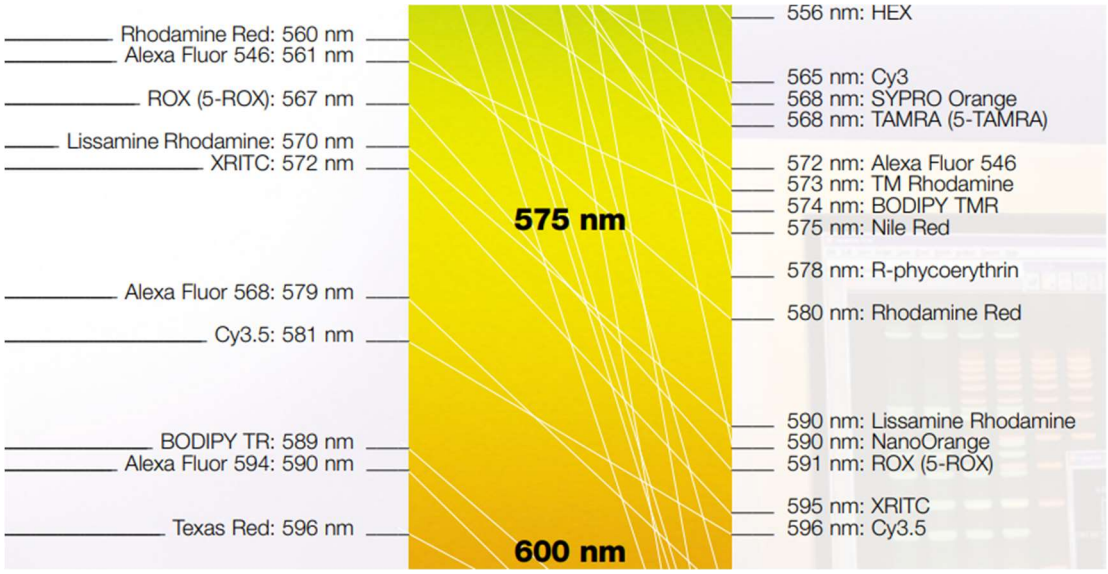
BIO-RAD EX.1009.073

Claims	Exemplary Disclosure of Accused Product
	wavelengths), is six. The number of analytes (e.g., targets) unambiguously determined, M, is twelve when the number of second values used to encode the analytes, C, is six. Twelve is greater than six, and twelve and six are both positive integers. According at least to the above examples, the processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software) makes determinations wherein M (e.g., twelve) is greater than the number of the second values used to encode the analytes (C) (e.g., six), and M and C are positive integers, both literally and under the doctrine of equivalents.
[2.pre] 2. The system of claim 1, wherein	Caltech incorporates the discussion from claim 1, *supra,* in its entirety. As discussed therein, the QX600 is a system comprising each and every element of claim 1, both literally and under the doctrine of equivalents.
[2.a.1] the multi-channel detector is further configured to detect a fifth electromagnetic signal at a fifth wavelength from the sample chamber, the fifth electromagnetic signal generated by excitement of a fifth fluorophore of the	The multi-channel detector is further configured to detect a fifth electromagnetic signal at a fifth wavelength from the sample chamber, the fifth electromagnetic signal generated by excitement of a fifth fluorophore of the multiple fluorophores, both literally and under the doctrine of equivalents. For example, the multi-channel detector (e.g., the QX600 Droplet Reader's six-color detector) detects electromagnetic signals in a ROX fluorophore emission wavelength after interrogating the droplet in a defined spectrum around the ROX fluorophore's excitation wavelength. *See, e.g.*:

BIO-RAD EX.1009.074

Claims	Exemplary Disclosure of Accused Product
multiple fluorophores:	 CT0479068 at CT0479090 The ROX fluorophore emits a fifth electromagnetic signal in a ROX fluorophore emission wavelength (e.g., 591 nm), upon excitation (e.g., interrogation) by the QX600 Droplet Reader. *See, e.g.*:

BIO-RAD EX.1009.075

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364 

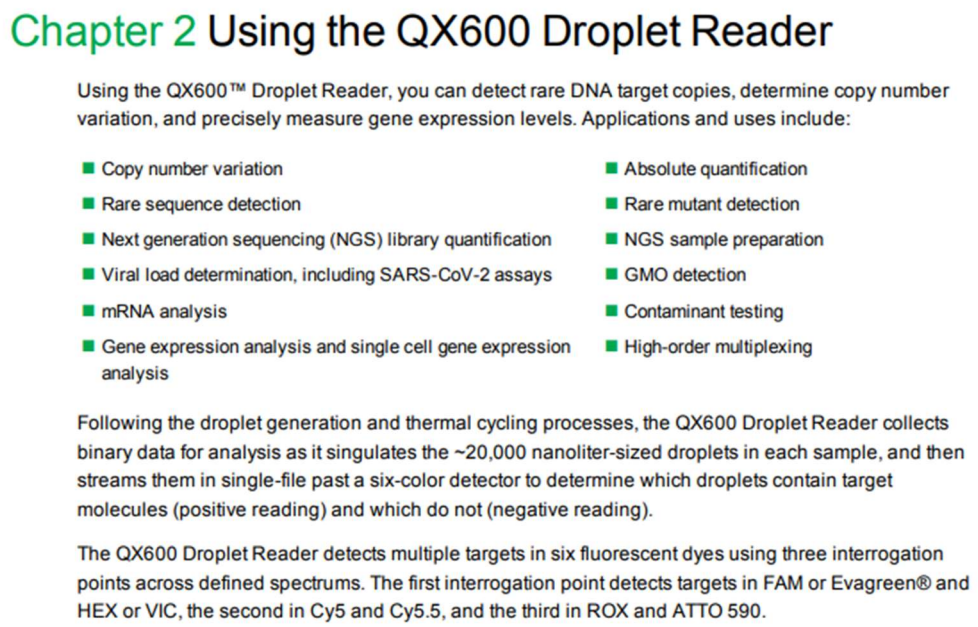
BIO-RAD EX.1009.076

Claims	Exemplary Disclosure of Accused Product
	CT0479364 at CT0479364 The ROX fluorophore is one of multiple fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590) that the QX600 Droplet Reader can detect after excitation (e.g., interrogation) at the given fluorophore excitation wavelength. *See, e.g.*: Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 According to at least the examples above, the multi-channel detector (e.g., the QX600 Droplet Reader's six-color detector) detects a fifth electromagnetic signal at a fifth wavelength (e.g., 591 nm) from the sample chamber (e.g., the 96-well plate), the fifth electromagnetic signal generated by

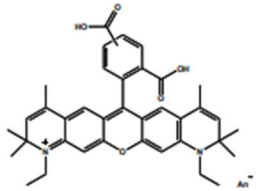
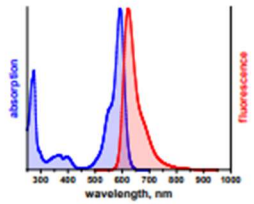
BIO-RAD EX.1009.077

Claims	Exemplary Disclosure of Accused Product
	excitement (e.g., interrogation) of a fifth fluorophore (e.g., the ROX fluorophore) of the multiple fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590), both literally and under the doctrine of equivalents. While ROX is designated here as the example fifth fluorophore and its emission wavelength is designated as the fifth wavelength, the fifth fluorophore can be any one of the FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590 fluorophores that is not designated as the first, second, third, fourth or sixth fluorophores, and the fifth wavelength is the emission wavelength of that fluorophore.
[2.a.2] a sixth electromagnetic signal at a sixth wavelength from the sample chamber, the sixth electromagnetic signal generated by excitement of a sixth fluorophore of the multiple fluorophores; and	The multi-channel detector is further configured to detect a sixth electromagnetic signal at a sixth wavelength from the sample chamber, the sixth electromagnetic signal generated by excitement of a sixth fluorophore of the multiple fluorophores, both literally and under the doctrine of equivalents. For example, the multi-channel detector (e.g., the QX600 Droplet Reader's six-color detector) detects electromagnetic signals in an ATTO 590 fluorophore emission wavelength after interrogating the droplet in a defined spectrum around the ATTO 590 fluorophore's excitation wavelength. *See, e.g.*:

BIO-RAD EX.1009.078

Claims	Exemplary Disclosure of Accused Product
	 Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 The ATTO 590 fluorophore emits a sixth electromagnetic signal in an ATTO 590 fluorophore emission wavelength (e.g., 622 nm), upon excitation (e.g., interrogation) by the QX600 Droplet Reader. *See, e.g.*:

BIO-RAD EX.1009.079

Claims	Exemplary Disclosure of Accused Product
	ATTO-TEC GmbH Martinshardt 7 D-57074 Siegen Germany *Phone:* +49 271 23853 – 0 *FAX:* +49 271 23853 – 11 *E-mail:* info@atto-tec.com *http:* www.atto-tec.com  ATTO-TEC *Revised:* 2023-11-06 **Product Information: ATTO 590**  **ATTO 590** is a novel fluorescent label belonging to the class of Rhodamine dyes. The dye is designed for application in the area of life science, e.g. labeling of DNA, RNA or proteins. Characteristic features of the label are strong absorption, high fluorescence quantum yield, high thermal and photo-stability, and very little triplet formation. The dye is highly suitable for single-molecule detection applications and high-resolution microscopy. As supplied **ATTO 590** consists of a mixture of two isomers with practically identical absorption and fluorescence. On request the dye can also be supplied as single isomer. ***Optical data of the carboxy derivative*** *(in PBS, pH 7.4):* λ_{abs} = 593 nm ε_{max} = 1.2 x 10^5 M^{-1} cm^{-1} λ_{fl} = 622 nm η_{fl} = 80 % τ_{fl} = 3.7 ns CF_{260} = 0.39 CF_{280} = 0.43   Spectra available in digitized form (excel file) on http://www.atto-tec.com CT0479362 at CT0479362 The ATTO 590 fluorophore is one of multiple fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590) that the QX600 Droplet Reader can detect after excitation (e.g., interrogation) at the given

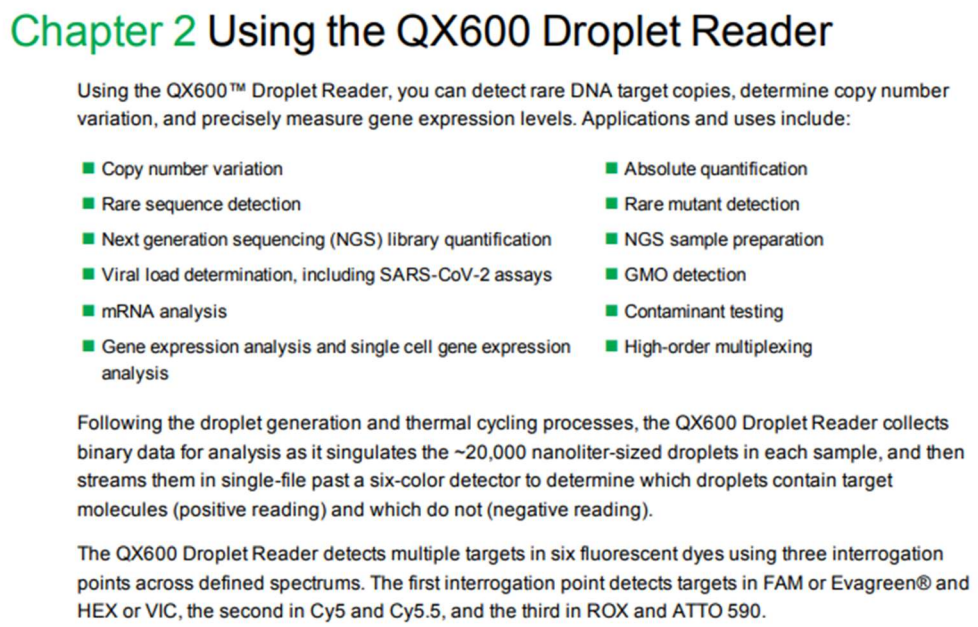
BIO-RAD EX.1009.080

Claims	Exemplary Disclosure of Accused Product
	fluorophore excitation wavelength. *See, e.g.*: Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 According to at least the examples above, the multi-channel detector (e.g., the QX600 Droplet Reader's six-color detector) detects a sixth electromagnetic signal at a sixth wavelength (e.g., 622 nm) from the sample chamber (e.g., the 96-well plate), the sixth electromagnetic signal generated by excitement (e.g., interrogation) of a sixth fluorophore (e.g., the ATTO 590 fluorophore) of the multiple fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590), both literally and under the doctrine of equivalents.

BIO-RAD EX.1009.081

Claims	Exemplary Disclosure of Accused Product
	While ATTO 590 is designated here as the example sixth fluorophore and its emission wavelength is designated as the sixth wavelength, the sixth fluorophore can be any one of the FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590 fluorophores that is not designated as the first, second, third, fourth or fifth fluorophores, and the sixth wavelength is the emission wavelength of that fluorophore.
[2.b] wherein C=6.	The processor controlled analyzer makes determinations wherein C= 6, both literally and under the doctrine of equivalents. For example, the QX600 Droplet Reader collects intensity data from up to six channels (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590), so the number of second values, C (e.g., fluorophore wavelengths) may be 1, 2, 3, 4, 5, or 6. *See, e.g.*:

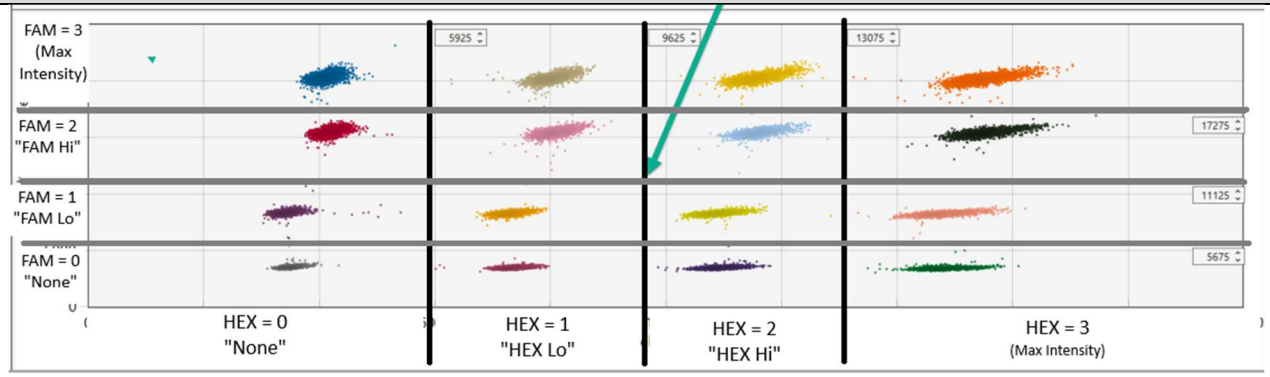
BIO-RAD EX.1009.082

Claims	Exemplary Disclosure of Accused Product
	 Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 According at least to the above examples, the processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software) makes determinations wherein C=6, both literally and under the doctrine of equivalents.

BIO-RAD EX.1009.083

Claims	Exemplary Disclosure of Accused Product
[5.pre] 5. The system of claim 1, wherein	Caltech incorporates the discussion from claim 1, *supra,* in its entirety. As discussed therein, the QX600 is a system comprising each and every element of claim 1, both literally and under the doctrine of equivalents.
[5.a] F=3.	The processor controlled analyzer makes determinations wherein F=3, both literally and under the doctrine of equivalents. For example, the processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software) applies a decoding matrix to the cumulative signal (e.g. assigns target analyte combinations to discrete signal channel levels and associates droplet data with target analyte combinations according to signal channel levels within the droplet data), such that maximum cumulative intensity for any channel, F, is three. *See, e.g.*:

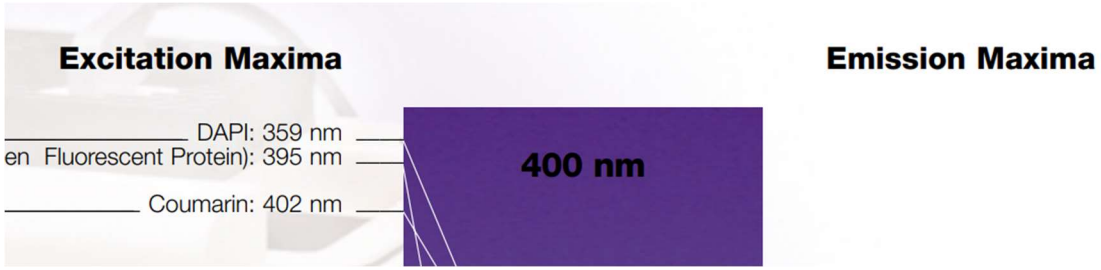
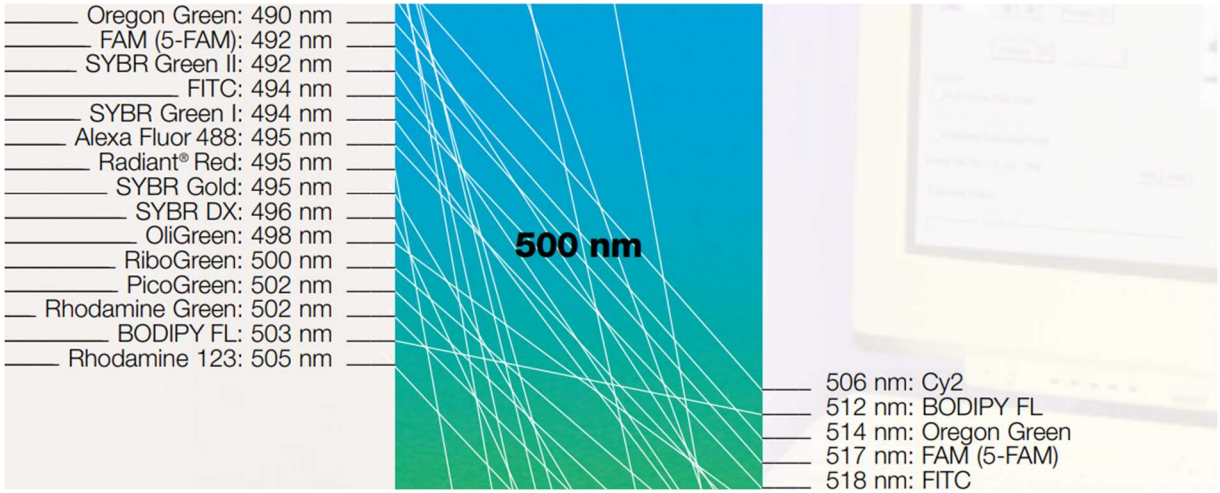
BIO-RAD EX.1009.084

Claims	Exemplary Disclosure of Accused Product
	 CT0479068 at CT0479252 (notation of discretized channel signal intensity levels added, threshold line emphasis added) According at least to the above examples, the processor controlled analyzer makes determinations wherein F=3, both literally and under the doctrine of equivalents.
[6.pre] 6. The system of claim 5, wherein	Caltech incorporates the discussion from claim 5, *supra,* in its entirety. As discussed therein, the QX600 is a system comprising each and every element of claim 5, both literally and under the doctrine of equivalents.
[6.a] the multiple fluorophores is selected from the group consisting of a blue	The QX600 detects electromagnetic waves at the emission wavelength of multiple fluorophores, wherein the multiple fluorophores is selected from the group consisting of a blue fluorophore, a green fluorophore, a yellow fluorophore, a red fluorophore, and any combinations thereof, both literally and under the doctrine of equivalents. For example, the QX600 detects electromagnetic waves at the emission wavelength of multiple

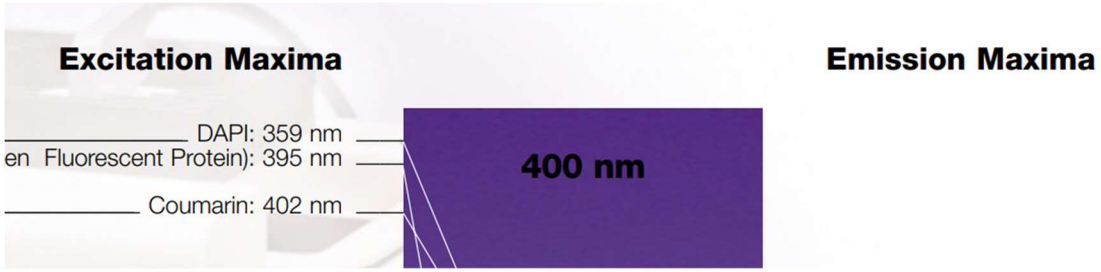
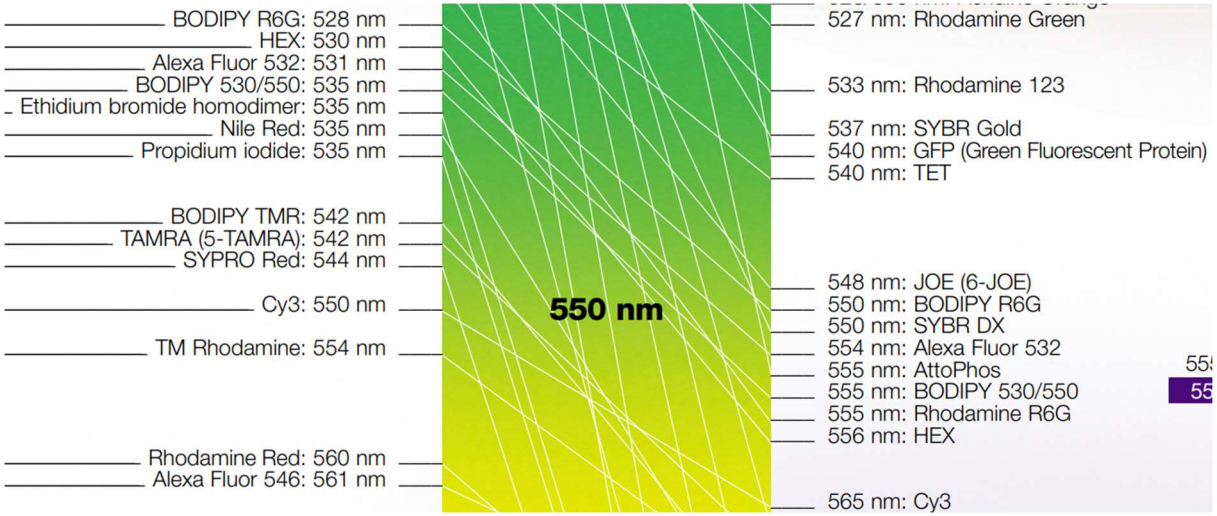
BIO-RAD EX.1009.085

Claims	Exemplary Disclosure of Accused Product
fluorophore, a green fluorophore, a yellow fluorophore, a red fluorophore, and any combinations thereof.	fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590). *See, e.g.*: Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 The FAM fluorophore is a green fluorophore. *See, e.g.*:

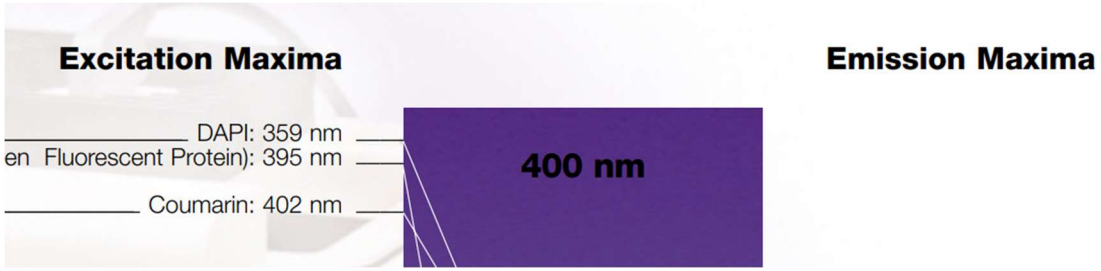
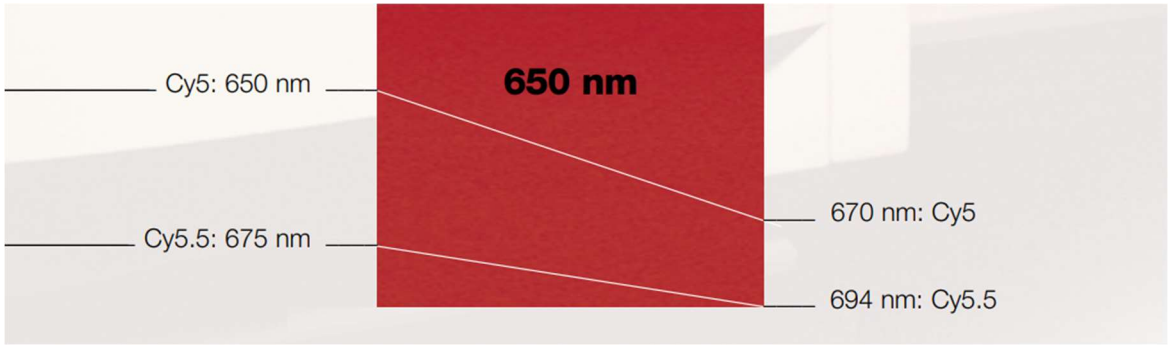
BIO-RAD EX.1009.086

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364  CT0479364 at CT0479364

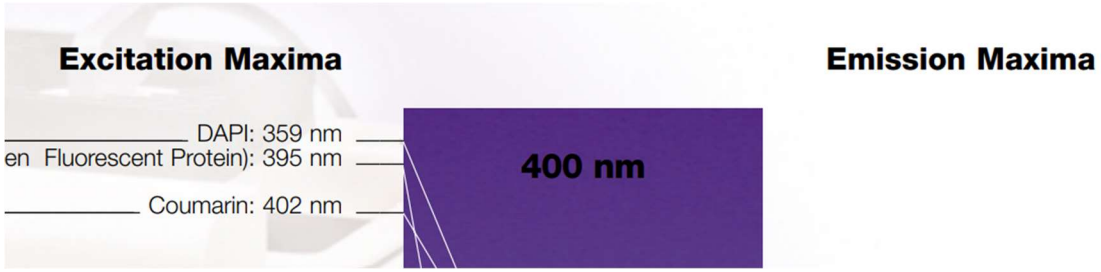
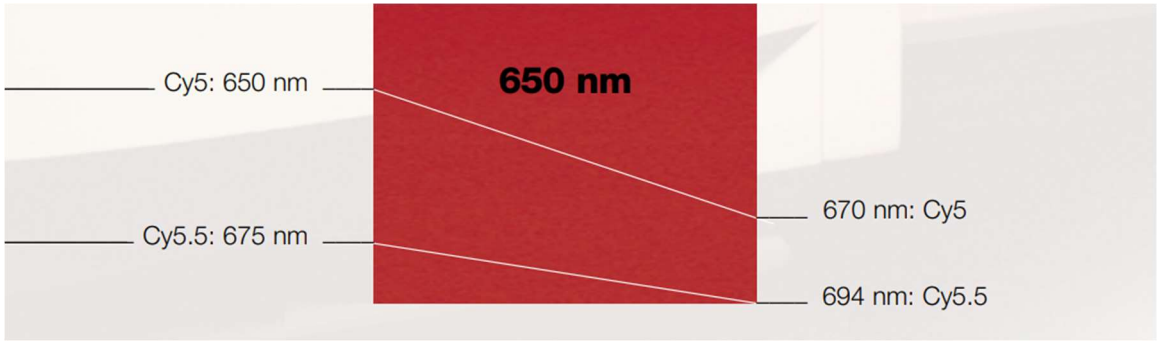
BIO-RAD EX.1009.087

Claims	Exemplary Disclosure of Accused Product
	The HEX fluorophore is a green and yellow fluorophore. *See, e.g.*:  CT0479364 at CT0479364  CT0479364 at CT0479364

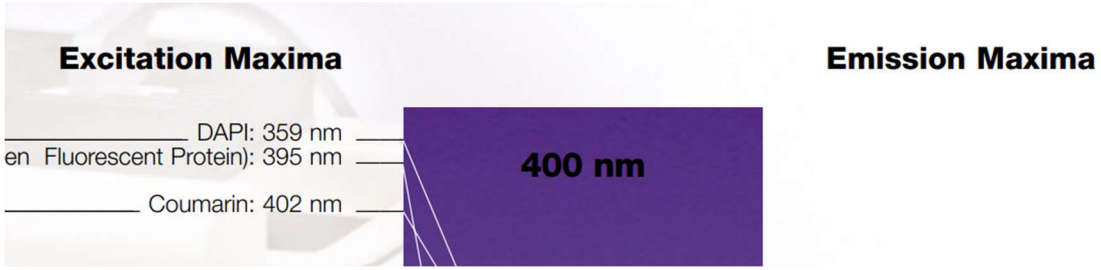
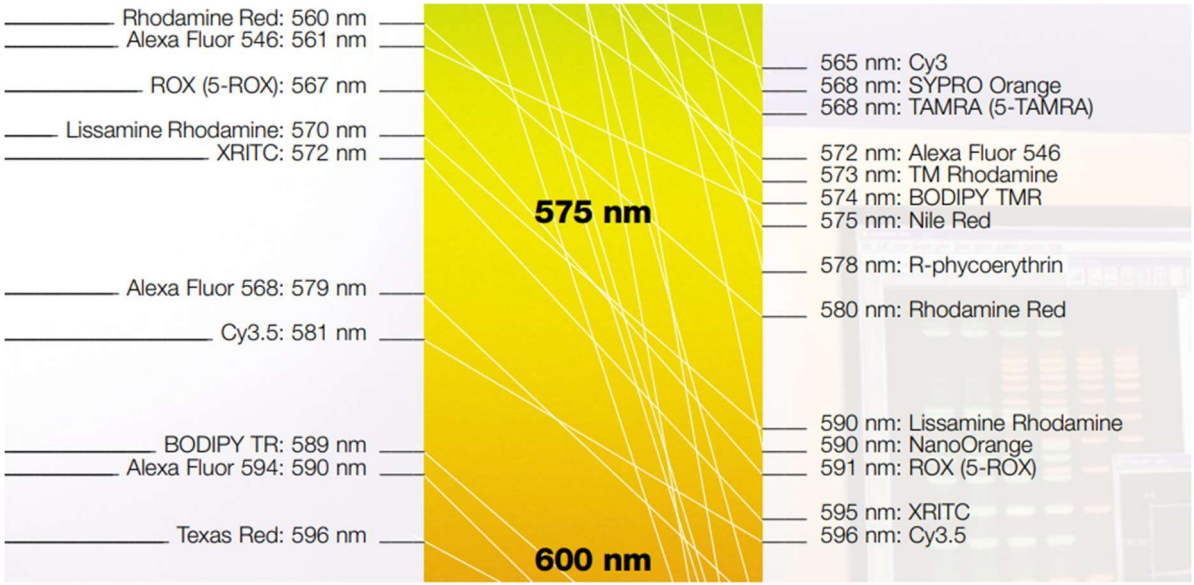
BIO-RAD EX.1009.088

Claims	Exemplary Disclosure of Accused Product
	The Cy5 fluorophore is a red fluorophore. *See, e.g.*:  CT0479364 at CT0479364  CT0479364 at CT0479364

BIO-RAD EX.1009.089

Claims	Exemplary Disclosure of Accused Product
	The Cy5.5 fluorophore is a red fluorophore. *See, e.g.*:  CT0479364 at CT0479364  CT0479364 at CT0479364 The ROX fluorophore is a yellow fluorophore. *See, e.g.*:

BIO-RAD EX.1009.090

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364  CT0479364 at CT0479364

BIO-RAD EX.1009.091

Claims	Exemplary Disclosure of Accused Product
	The ATTO 590 fluorophore has an excitation wavelength of about 593 nm and an emission wavelength of about 622 nm. *See, e.g.*:

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FAX: +49 271 23853 – 11
E-mail: info@atto-tec.com
http: www.atto-tec.com



ATTO-TEC

Revised: 2023-11-06

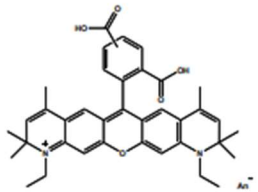
Product Information: ATTO 590

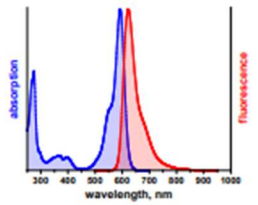


ATTO 590 is a novel fluorescent label belonging to the class of Rhodamine dyes. The dye is designed for application in the area of life science, e.g. labeling of DNA, RNA or proteins. Characteristic features of the label are strong absorption, high fluorescence quantum yield, high thermal and photo-stability, and very little triplet formation. The dye is highly suitable for single-molecule detection applications and high-resolution microscopy.
As supplied **ATTO 590** consists of a mixture of two isomers with practically identical absorption and fluorescence. On request the dye can also be supplied as single isomer.

***Optical data of the carboxy derivative** (in PBS, pH 7.4):*

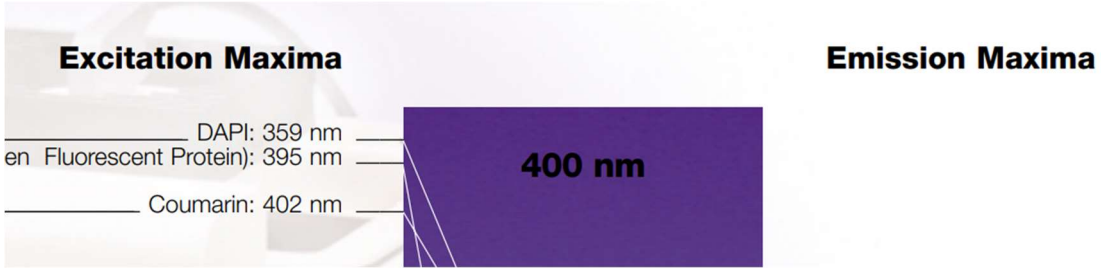
λ_{abs}	=	593 nm
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}
λ_{fl}	=	622 nm
η_{fl}	=	80 %
τ_{fl}	=	3.7 ns
CF_{260}	=	0.39
CF_{280}	=	0.43



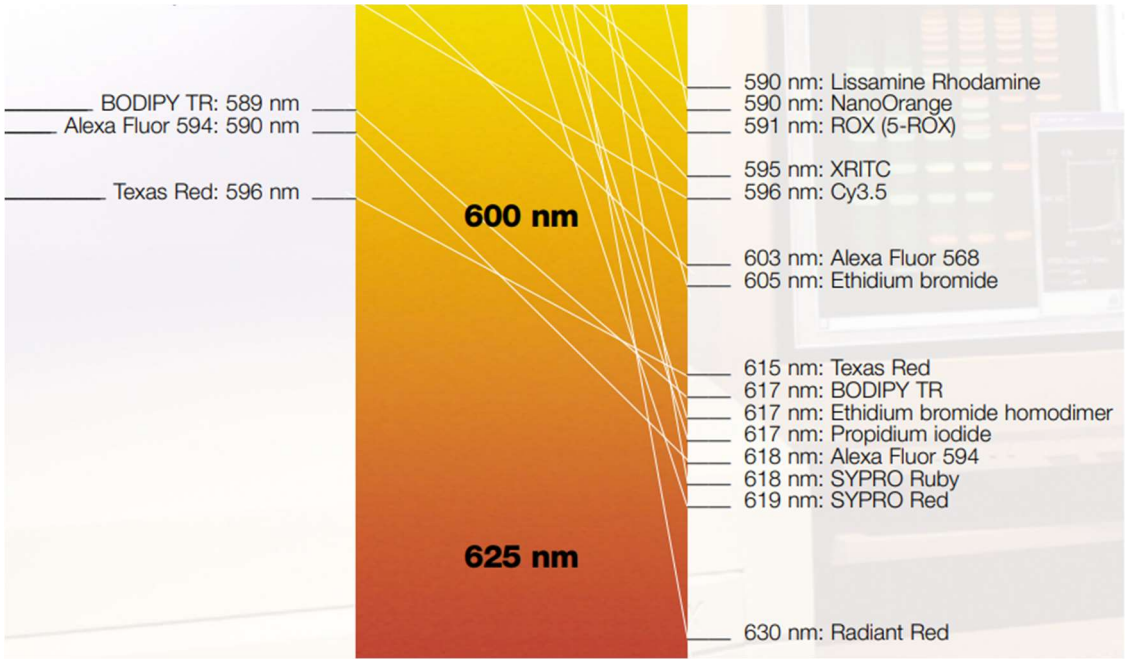


Spectra available in digitized form (excel file) on
http://www.atto-tec.com

BIO-RAD EX.1009.092

Claims	Exemplary Disclosure of Accused Product
	CT0479362 at CT0479362 The ATTO 590 fluorophore's emission wavelength of about 622 nm makes ATTO 590 a red fluorophore. *See, e.g.*:  CT0479364 at CT0479364

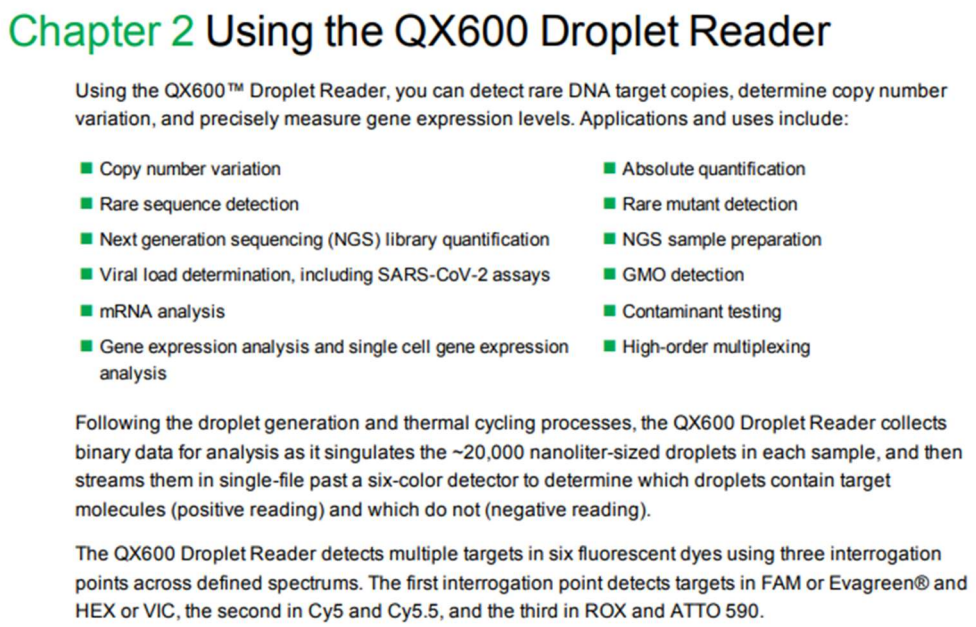
BIO-RAD EX.1009.093

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364 According to at least the above examples, the QX600 detects electromagnetic waves at the emission wavelength of multiple fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590), wherein the multiple fluorophores is selected from the group consisting of a blue fluorophore, a green fluorophore (e.g., FAM), a yellow fluorophore (e.g., ROX), a red fluorophore (e.g., Cy5, Cy5.5, ATTO 590), and any combinations thereof (e.g., HEX which is green and yellow), both literally and under the doctrine of equivalents.

BIO-RAD EX.1009.094

Claims	Exemplary Disclosure of Accused Product
[7.pre] 7. The system of claim 5, wherein	Caltech incorporates the discussion from claim 5, *supra,* in its entirety. As discussed therein, the QX600 is a system comprising each and every element of claim 5, both literally and under the doctrine of equivalents.
[7.a] the multiple fluorophores is selected from the group consisting of a fluorescein amidite (FAM), a cyanine 3 (Cy3), a carboxy-X-rhodamine (ROX), a cyanine 5 (Cy5), a cyanine 5.5 (Cy5.5), and any combinations thereof.	The QX600 detects electromagnetic waves at the emission wavelength of multiple fluorophores, wherein the multiple fluorophores is selected from the group consisting of a fluorescein amidite (FAM), a cyanine 3 (Cy3), a carboxy-X-rhodamine (ROX), a cyanine 5 (Cy5), a cyanine 5.5 (Cy5.5), and any combinations thereof, both literally and under the doctrine of equivalents. For example, the QX600 detects electromagnetic waves at the emission wavelength of multiple fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590). *See, e.g.*:

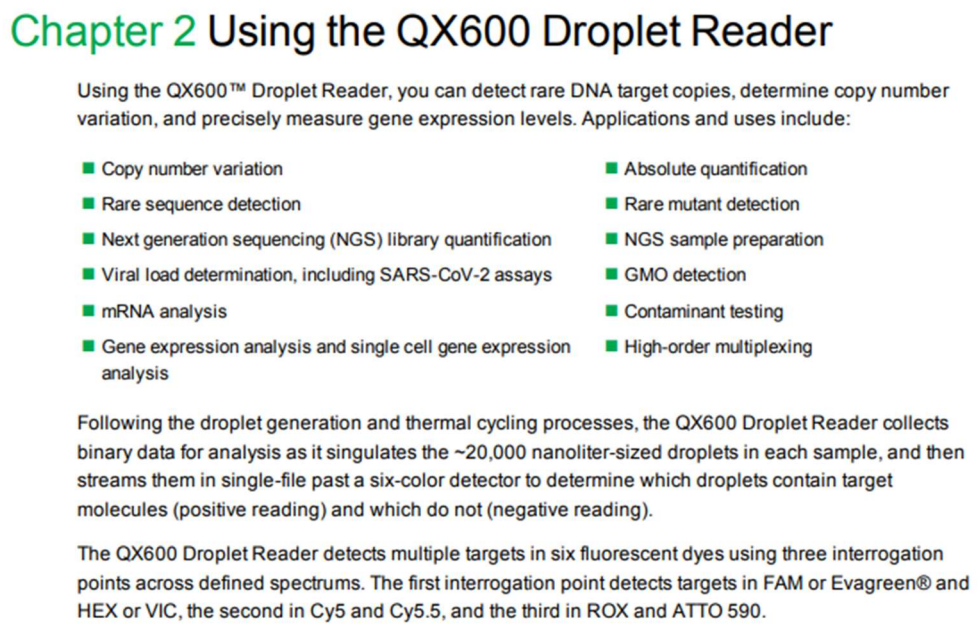
BIO-RAD EX.1009.095

Claims	Exemplary Disclosure of Accused Product
	 CT0479068 at CT0479090 According to at least the above examples, the QX600 detects electromagnetic waves at the emission wavelength of multiple fluorophores (e.g., FAM, ROX, Cy5, Cy5.5, ROX, and ATTO 590), wherein the multiple fluorophores is selected from the group consisting of a fluorescein amidite (FAM), a cyanine 3 (Cy3), a carboxy-X-rhodamine (ROX), a cyanine 5 (Cy5), a cyanine 5.5 (Cy5.5), and any combinations thereof, both literally and under the doctrine of equivalents.

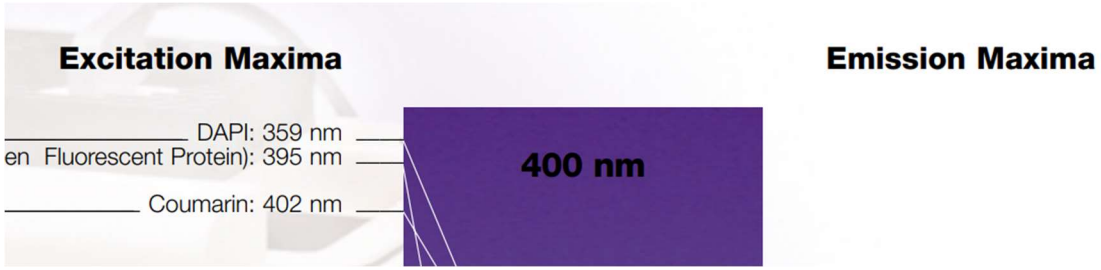
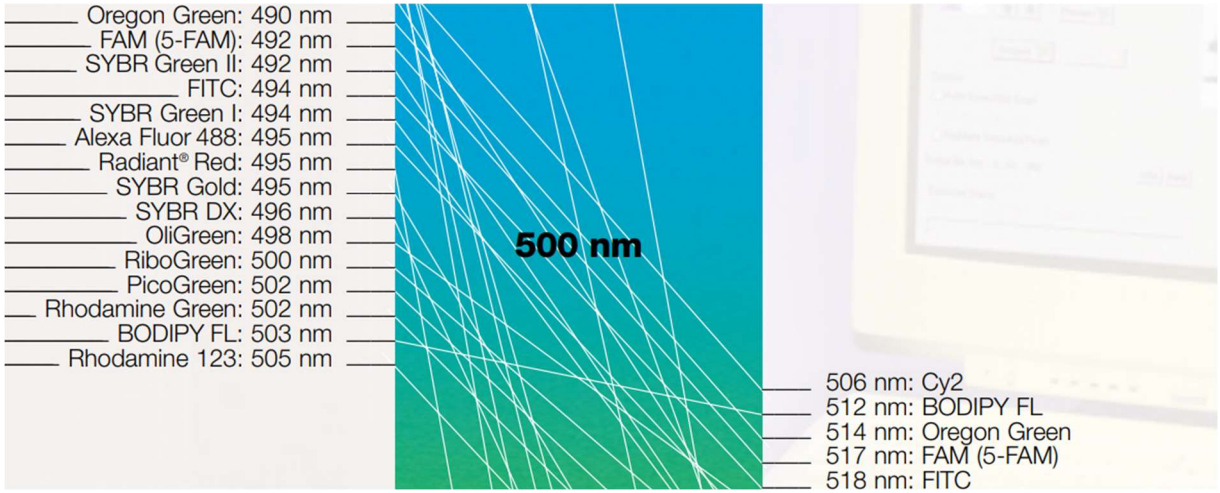
BIO-RAD EX.1009.096

Claims	Exemplary Disclosure of Accused Product
[8.pre] 8. The system of claim 5, wherein	Caltech incorporates the discussion from claim 5, *supra,* in its entirety. As discussed therein, the QX600 is a system comprising each and every element of claim 5, both literally and under the doctrine of equivalents.
[8.a] the multiple fluorophores has a maximum excitation wavelength selected from the group consisting of about 494 nm, about 550 nm, about 567 nm, about 650 nm, about 675 nm, and any combinations thereof.	The QX600 detects electromagnetic waves at the emission wavelength of multiple fluorophores, wherein the multiple fluorophores has a maximum excitation wavelength selected from the group consisting of about 494 nm, about 550 nm, about 567 nm, about 650 nm, about 675 nm, and any combinations thereof, both literally and under the doctrine of equivalents. For example, the QX600 detects electromagnetic waves at the emission wavelength of multiple fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590). *See, e.g.*:

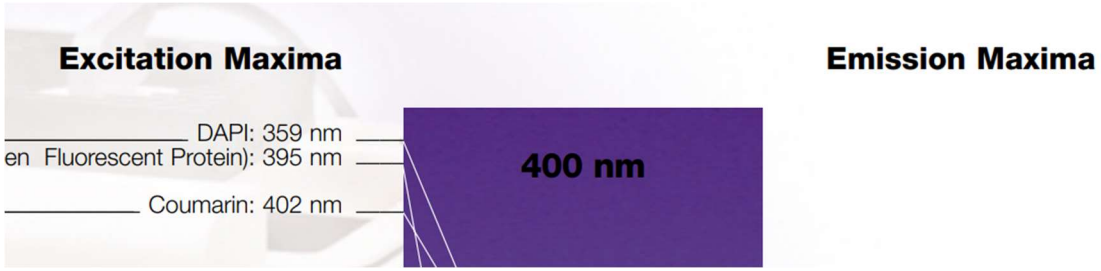
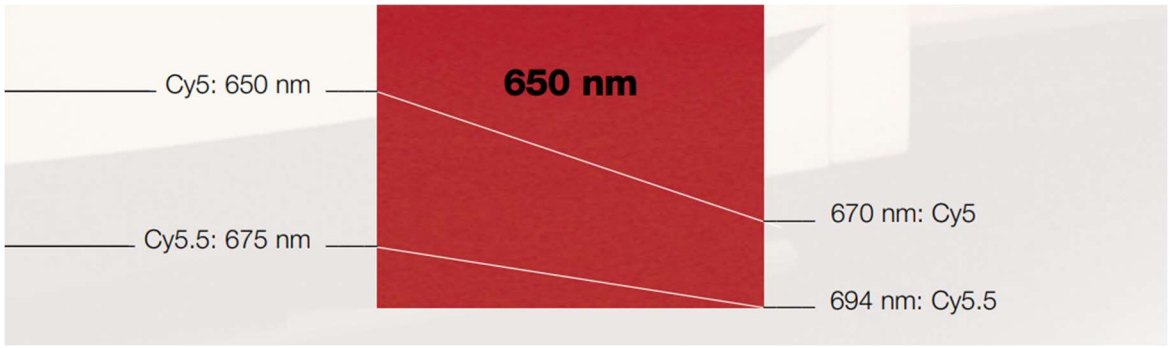
BIO-RAD EX.1009.097

Claims	Exemplary Disclosure of Accused Product
	 Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 The FAM fluorophore has a maximum excitation wavelength of about 494 nm (e.g., 492 nm). *See, e.g.*:

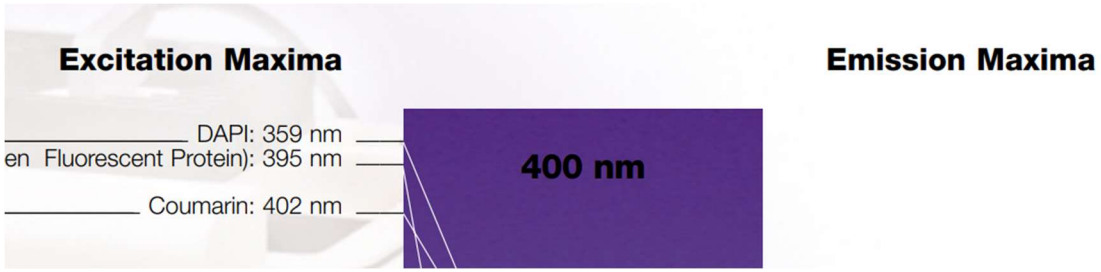
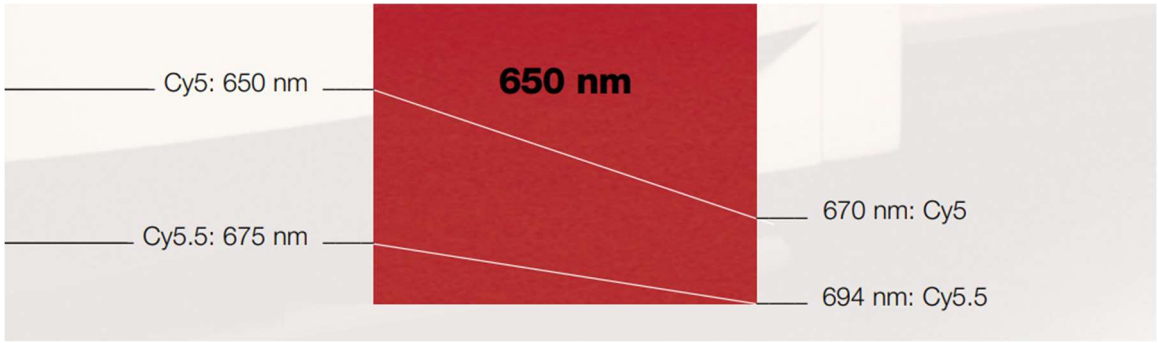
BIO-RAD EX.1009.098

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364  CT0479364 at CT0479364

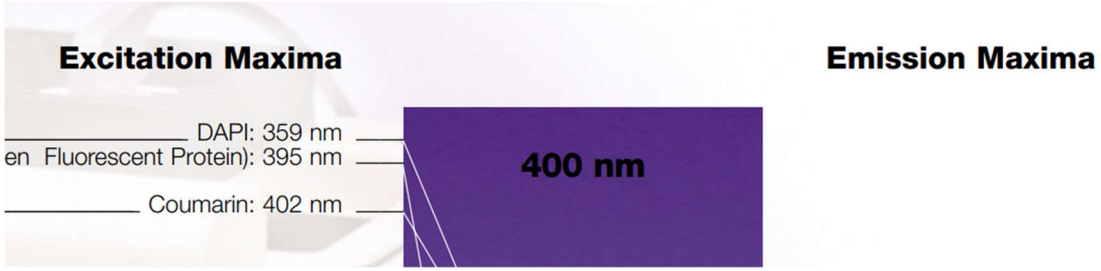
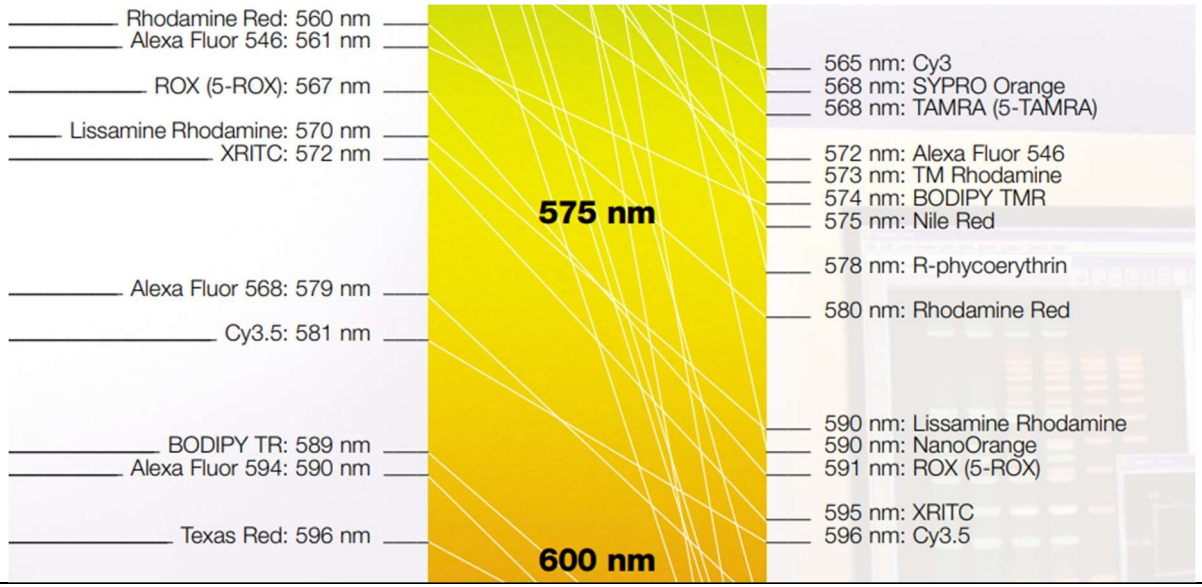
BIO-RAD EX.1009.099

Claims	Exemplary Disclosure of Accused Product
	The Cy5 fluorophore has a maximum excitation wavelength of about 650 nm (e.g., 650 nm). *See, e.g.*:  CT0479364 at CT0479364  CT0479364 at CT0479364

BIO-RAD EX.1009.100

Claims	Exemplary Disclosure of Accused Product
	The Cy5.5 fluorophore has a maximum excitation wavelength of about 675 nm (e.g., 675 nm). *See, e.g.*:  CT0479364 at CT0479364  CT0479364 at CT0479364 The ROX fluorophore has a maximum excitation wavelength of about 567 nm (e.g., 567 nm). *See,*

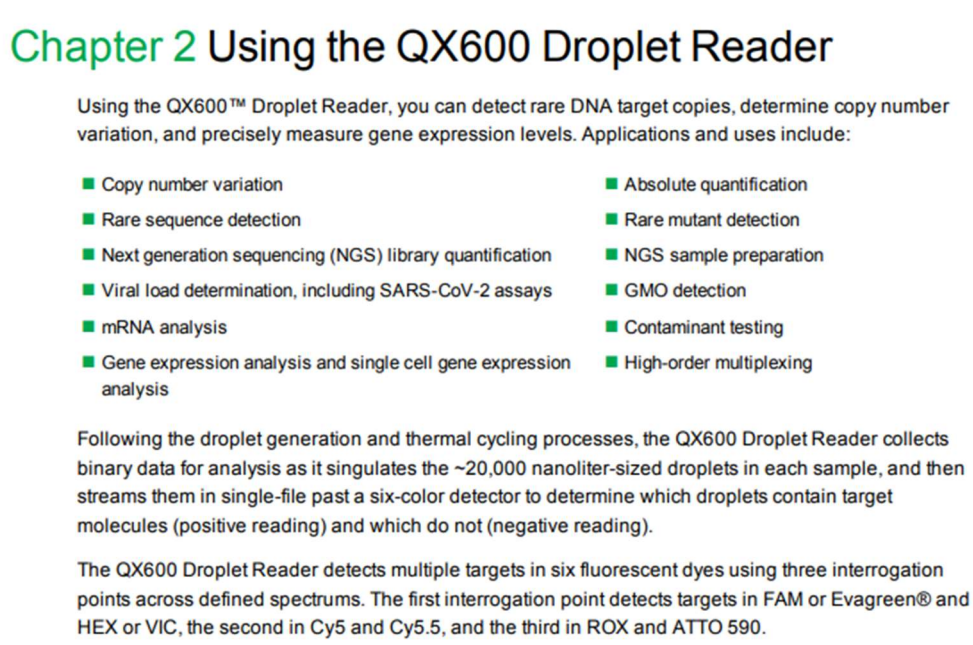
BIO-RAD EX.1009.101

Claims	Exemplary Disclosure of Accused Product
	e.g.:  CT0479364 at CT0479364 

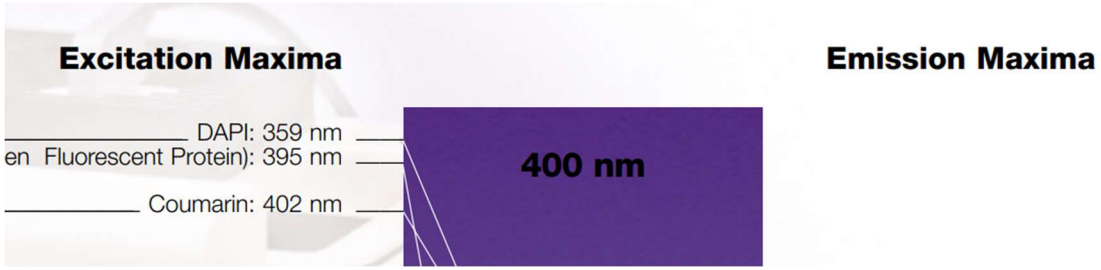
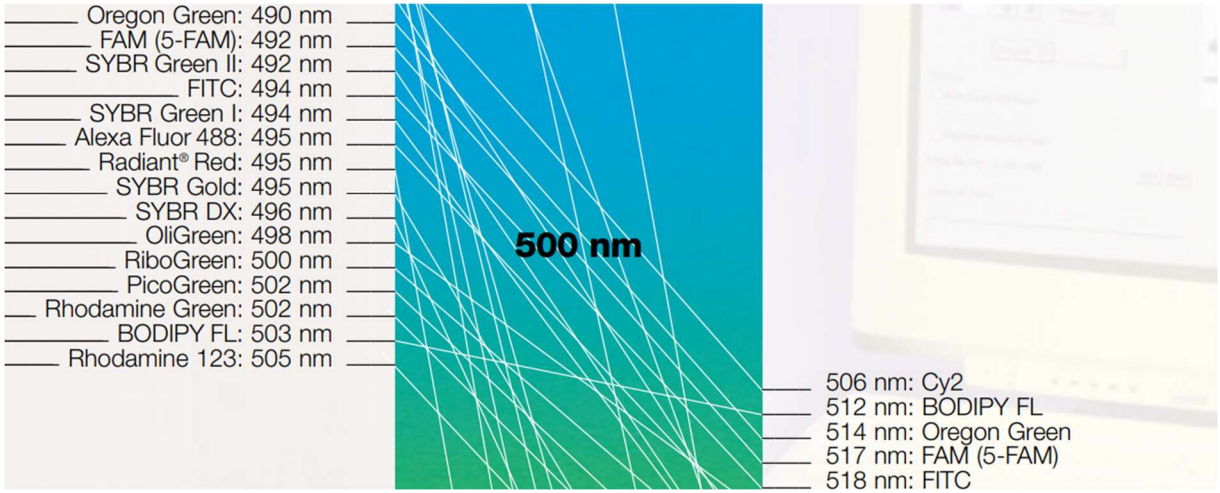
BIO-RAD EX.1009.102

Claims	Exemplary Disclosure of Accused Product
	CT0479364 at CT0479364 According to at least the above examples, the QX600 detects electromagnetic waves at the emission wavelength of multiple fluorophores (e.g., FAM, Cy5, Cy5.5, and ROX), wherein the multiple fluorophores has a maximum excitation wavelength selected from the group consisting of about 494 nm (e.g., FAM: 492 nm), about 567 nm (e.g., ROX: 567 nm), about 650 nm (e.g., Cy5: 650 nm), about 675 nm (e.g., Cy5.5: 675 nm), and any combinations thereof, both literally and under the doctrine of equivalents.
[9.pre] 9. The system of claim 5, wherein	Caltech incorporates the discussion from claim 5, *supra,* in its entirety. As discussed therein, the QX600 is a system comprising each and every element of claim 5, both literally and under the doctrine of equivalents.
[9.a] the multiple fluorophores has a maximum emission wavelength selected from the group	The QX600 detects electromagnetic waves at the emission wavelength of multiple fluorophores, wherein the multiple fluorophores has a maximum emission wavelength selected from the group consisting of about 518 nm, about 565 nm, about 591 nm, about 670 nm, about 697 nm, and any combinations thereof, both literally and under the doctrine of equivalents. For example, the QX600 detects electromagnetic waves at the emission wavelength of multiple fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590). *See, e.g.*:

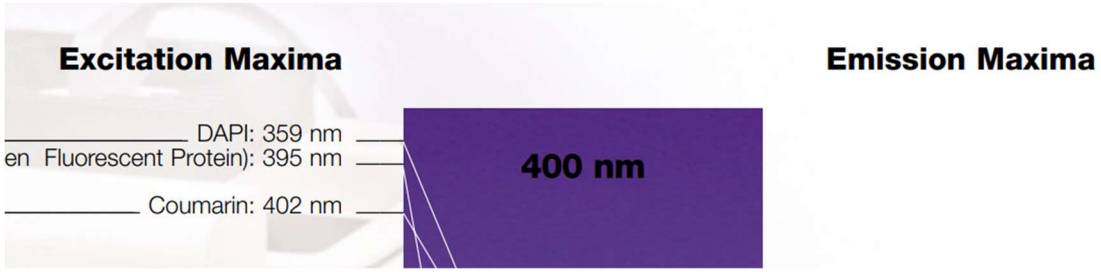
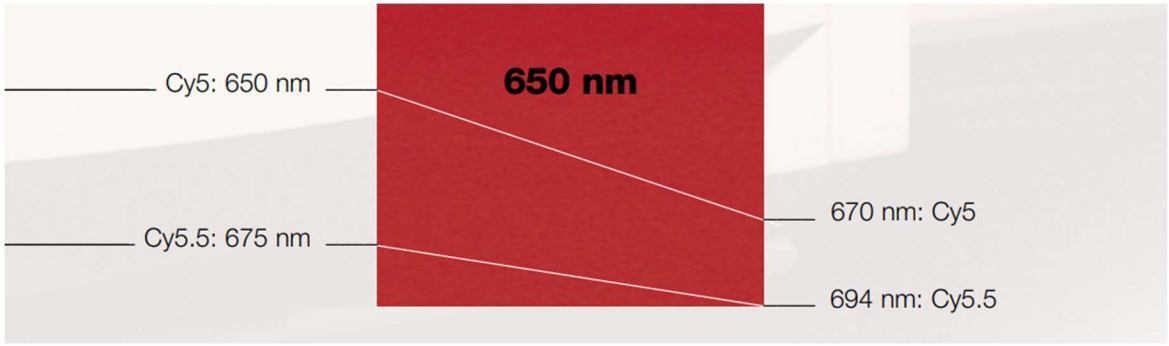
BIO-RAD EX.1009.103

Claims	Exemplary Disclosure of Accused Product
consisting of about 518 nm, about 565 nm, about 591 nm, about 670 nm, about 697 nm, and any combinations thereof.	 Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 The FAM fluorophore has a maximum emission wavelength of about 518 nm (e.g., 517 nm). *See, e.g.*:

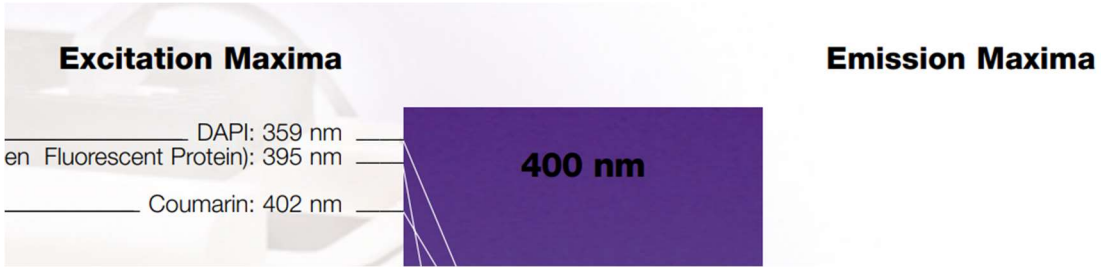
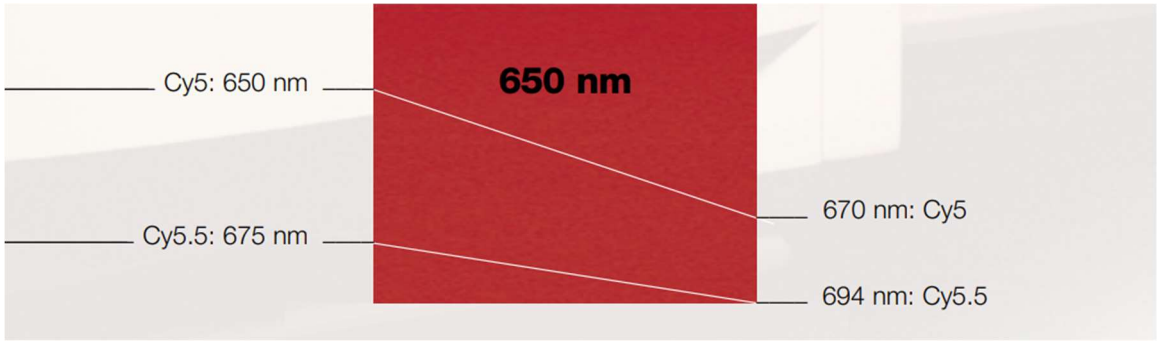
BIO-RAD EX.1009.104

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364  CT0479364 at CT0479364

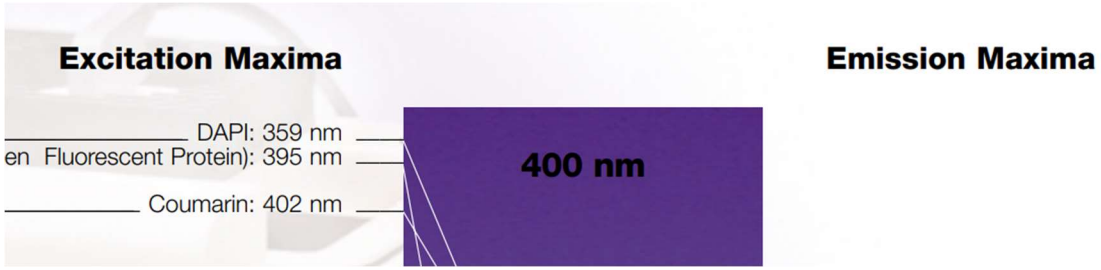
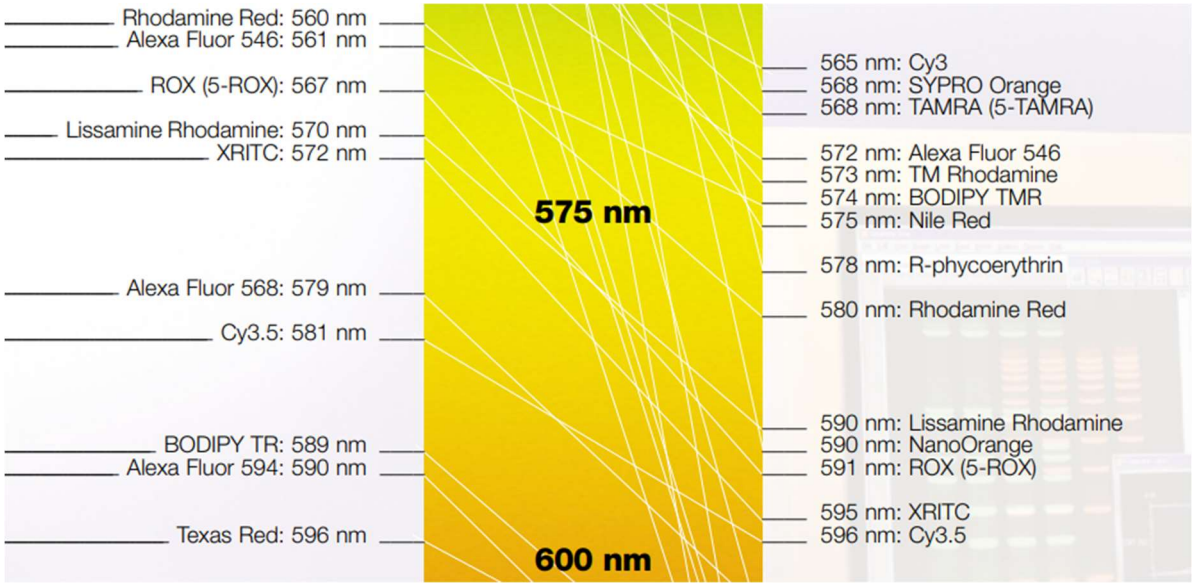
BIO-RAD EX.1009.105

Claims	Exemplary Disclosure of Accused Product
	The Cy5 fluorophore has a maximum emission wavelength of about 670 nm (e.g., 670 nm). *See, e.g.*:  CT0479364 at CT0479364  CT0479364 at CT0479364 The Cy5.5 fluorophore has a maximum emission wavelength of about 697 nm (e.g., 694 nm). *See, e.g.*:

BIO-RAD EX.1009.106

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364  CT0479364 at CT0479364 The ROX fluorophore has a maximum emission wavelength of about 591 nm (e.g., 591 nm). *See, e.g.*:

BIO-RAD EX.1009.107

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364  CT0479364 at CT0479364

BIO-RAD EX.1009.108

Claims	Exemplary Disclosure of Accused Product
	According to at least the above examples, the QX600 detects electromagnetic waves at the emission wavelength of multiple fluorophores (e.g., FAM, Cy5, Cy5.5, and ROX), wherein the multiple fluorophores has a maximum emission wavelength selected from the group consisting of about 518 nm (e.g., FAM: 517 nm), about 591 nm (e.g., ROX: 591 nm), about 670 nm (e.g., Cy5: 670 nm), about 697 nm (e.g., Cy5.5: 694 nm), and any combinations thereof, both literally and under the doctrine of equivalents.
[10.pre] 10. The system of claim 5, wherein	Caltech incorporates the discussion from claim 5, *supra,* in its entirety. As discussed therein, the QX600 is a system comprising each and every element of claim 5, both literally and under the doctrine of equivalents.
[10.a] the analyte-specific hybridization probes are labeled with a fluorophore of the multiple fluorophores.	The analyte-specific hybridization probes within the analyte-specific reagent mixture are labeled with a fluorophore of the multiple fluorophores, both literally and under the doctrine of equivalents. For example, the QX600 ddPCR workflow includes combining a sample (e.g., DNA or RNA) with an analyte-specific reagent mixture (e.g., primers, probes, and Bio-Rad ddPCR supermix). *See, e.g.*:

BIO-RAD EX.1009.109

Claims	Exemplary Disclosure of Accused Product
	ddPCR Workflow The ddPCR process adheres to the following workflow: ■ You prepare your samples for PCR by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermix. ■ A droplet generator fractionates a sample into~20,000 nanoliter-sized droplets with target and background DNA distributed randomly into the droplets during the partitioning process. ■ Following droplet generation, the droplets are run through a thermal cycler, which performs PCR amplification of the nucleic acid target in each individual droplet. ■ A droplet reader reads each droplet to determine the fraction of positive droplets in the original sample and uses Poisson statistical formulas to determine the absolute quantity. **Note:** Positive droplets containing at least one copy of the target DNA molecule exhibit increased fluorescence compared to negative droplets. CT0479068 at CT0479089 The analyte-specific reagent mixtures (e.g. primers, probes, and Bio-Rad ddPCR supermix) include certain PCR supermixes for probe-based assays, including hydrolysis probe-based assays. *See, e.g.*:

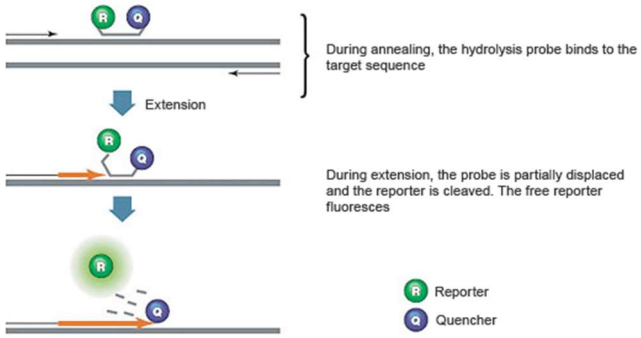
BIO-RAD EX.1009.110

Claims	Exemplary Disclosure of Accused Product
	Supermixes QX Manager Software offers five PCR supermixes. Each supermix is optimized to deliver maximum PCR efficiency and sensitivity for the amplification and detection of DNA and RNA targets. **Important:** Use of unapproved supermixes can harm the instrument and void the warranty. **ddPCR Supermix** / **Description** Multiplex Supermix — For use in nucleic acid sample preparations to amplify and detect DNA targets in probe-based experiments involving multiple targets. **Note:** Bio-Rad recommends using ddPCR Multiplex Supermix for Cy5 and Cy5.5 probes assays. Supermix for Probes (No dUTP) — For use in nucleic acid sample preparations, with sensitivity for the amplification and detection of DNA targets using hydrolysis probe-based assays. Supermix for Probes — For use in sample preparation with uracil N-glucosylase (UNG) decontamination protocols to prevent the reamplification of carryover PCR products between experiments. Supermix for Residual DNA Quantification — For use in residual DNA detection. One-Step RT Advanced Kit for Probe — For use in absolute quantification of target RNA molecules. EvaGreen® Supermix — For use in gene expression. CT0479068 at CT0479138

BIO-RAD EX.1009.111

Claims	Exemplary Disclosure of Accused Product
	Hydrolysis probes are labeled with a fluorophore (e.g. a reporter) of the multiple fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, ATTO 590). *See, e.g.*:

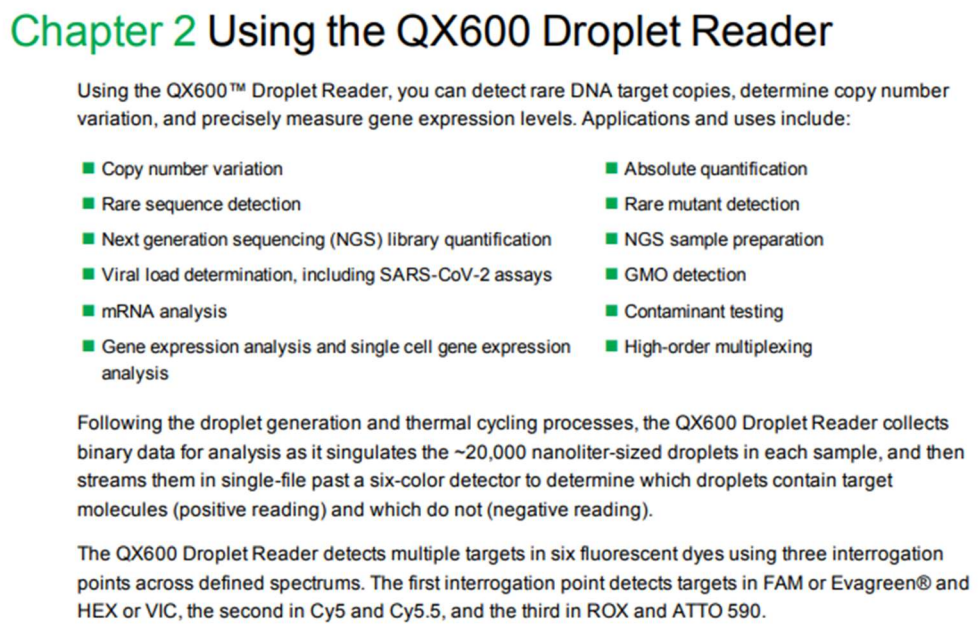
BIO-RAD EX.1009.112

Claims	Exemplary Disclosure of Accused Product
	Hydrolysis (TaqMan) Probes Hydrolysis assays (TaqMan or 5' nuclease assays) include a sequence-specific, fluorescently labeled oligonucleotide probe in addition to a sequence-specific PCR primer. Hydrolysis assays exploit the 5' → 3' exonuclease activity of certain thermostable polymerases such as *Taq* or *Tth*. The hydrolysis probe is labeled with a fluorescent reporter at the 5' end and a quencher at the 3' end. Commonly used fluorescent reporter-quencher pairs are fluorescein (FAM), which emits green fluorescence, and Black Hole Quencher 1 dye.  **Fig. 2. Hydrolysis probes are hydrolyzed by *Taq* polymerase.** When the hydrolysis probe is intact, the fluorescence of the reporter is quenched due to its proximity to the quencher (Figure 2). The amplification reaction includes a combined annealing/extension step during which the probe hybridizes to the target, and the dsDNA-specific 5' → 3' exonuclease activity of *Taq* or *Tth* cleaves off the reporter. As a result, the reporter is separated from the quencher, resulting in a fluorescence signal that is proportional to the amount of amplified product in the sample. The main advantages of using hydrolysis probes are high specificity, a high signal-to-noise ratio, and the ability to perform multiplex reactions. The disadvantages are that the initial cost of the probe may be high, and the assay design may not be trivial. CT0481963 at CT0481965–CT0481966

BIO-RAD EX.1009.113

Claims	Exemplary Disclosure of Accused Product
	According to at least the above examples, the analyte-specific hybridization probes (e.g., hydrolysis probes) within the analyte-specific reagent mixture are labeled with a fluorophore (e.g., a reporter) of the multiple fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, ATTO 590), both literally and under the doctrine of equivalents.
[11.pre] 11. The system of claim 5, wherein	Caltech incorporates the discussion from claim 5, *supra,* in its entirety. As discussed therein, the QX600 is a system comprising each and every element of claim 5, both literally and under the doctrine of equivalents.
[11.a] the detector comprises four or six band pass filters.	The multi-channel detector within QX600 comprises four or six band pass filters, both literally and under the doctrine of equivalents. For example, the QX600 detects electromagnetic waves at the emission wavelength of six fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590). *See, e.g.*:

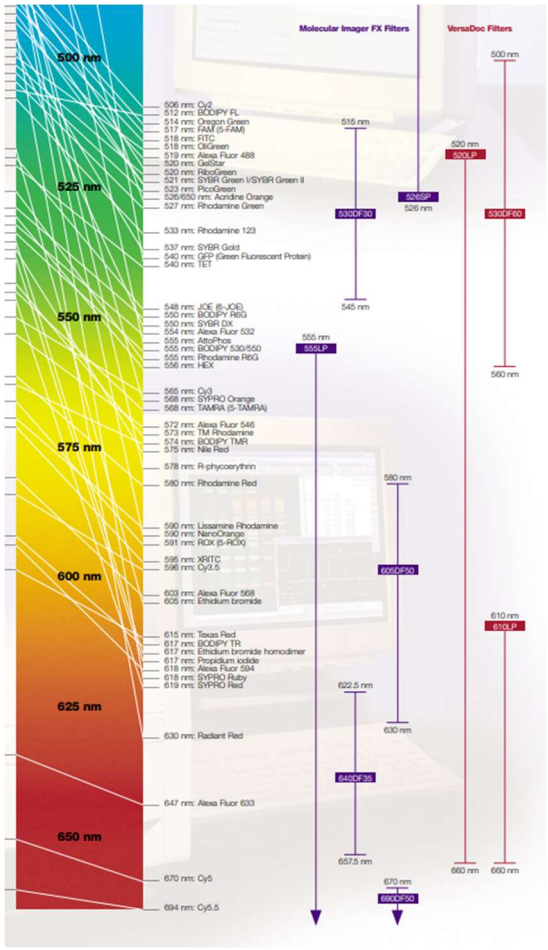
BIO-RAD EX.1009.114

Claims	Exemplary Disclosure of Accused Product
	 Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 On information and belief, the QX600 comprises at least six band-pass filters to filter received electromagnetic signals for signals with wavelengths near the emission wavelengths of the six fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590). *See, e.g.* (band pass ("DF") filters utilized in Bio-Rad products listed with filter ranges around the emission wavelengths of fluorophores):

BIO-RAD EX.1009.115

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364

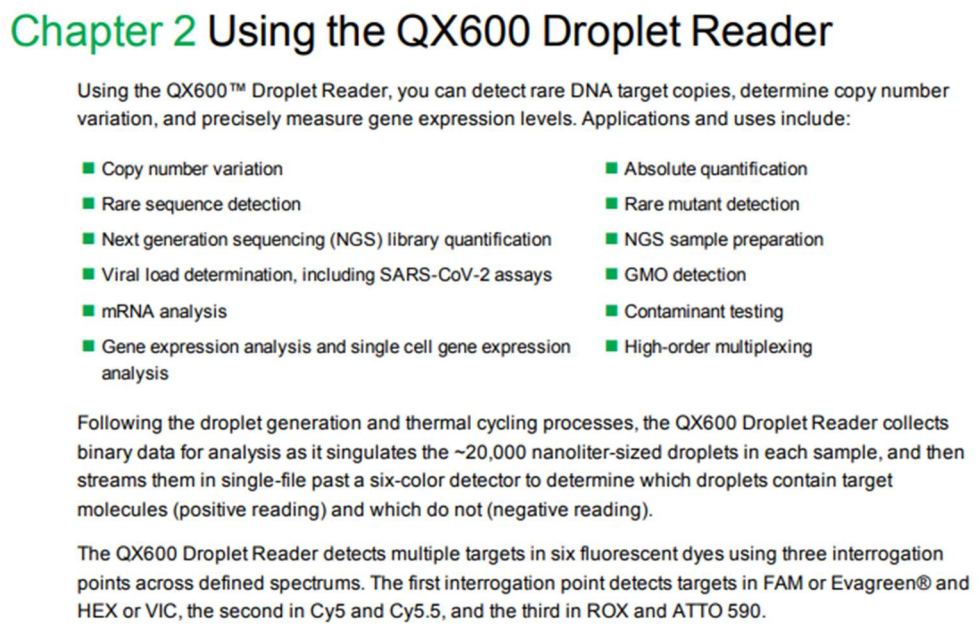
BIO-RAD EX.1009.116

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364

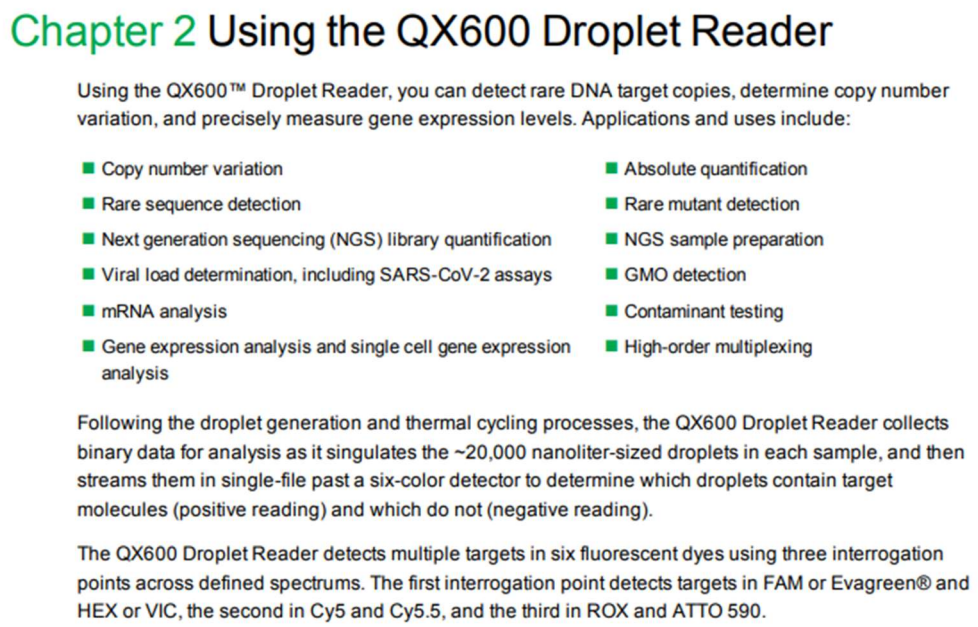
BIO-RAD EX.1009.117

Claims	Exemplary Disclosure of Accused Product
	According to at least the above examples, the multi-channel detector within QX600 comprises four or six band pass filters (e.g., six), both literally and under the doctrine of equivalents.
[13.pre] 13. The system of claim 5, wherein	Caltech incorporates the discussion from claim 5, *supra,* in its entirety. As discussed therein, the QX600 is a system comprising each and every element of claim 5, both literally and under the doctrine of equivalents.
[13.a] the multi-channel detector comprises four or six photodetectors.	The multi-channel detector within QX600 comprises four or six photodetectors, both literally and under the doctrine of equivalents. For example, the QX600 detects electromagnetic waves at the emission wavelength of six fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590). *See, e.g.*:

BIO-RAD EX.1009.118

Claims	Exemplary Disclosure of Accused Product
	 CT0479068 at CT0479090 On information and belief, the QX600 comprises photodetectors capable of detecting electromagnetic signals with wavelengths near the emission wavelengths of the six fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590). *See, e.g.* (QX600 Droplet Reader detects using three interrogation points across defined spectrums for each of the six fluorophores):

BIO-RAD EX.1009.119

Claims	Exemplary Disclosure of Accused Product
	 Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 According to at least the examples above, the multi-channel detector within QX600 comprises four or six photodetectors (e.g., six photodetectors), both literally and under the doctrine of equivalents.
[14.pre] 14. The system of claim 5, wherein	Caltech incorporates the discussion from claim 5, *supra,* in its entirety. As discussed therein, the QX600 is a system comprising each and every element of claim 5, both literally and under the doctrine of equivalents.

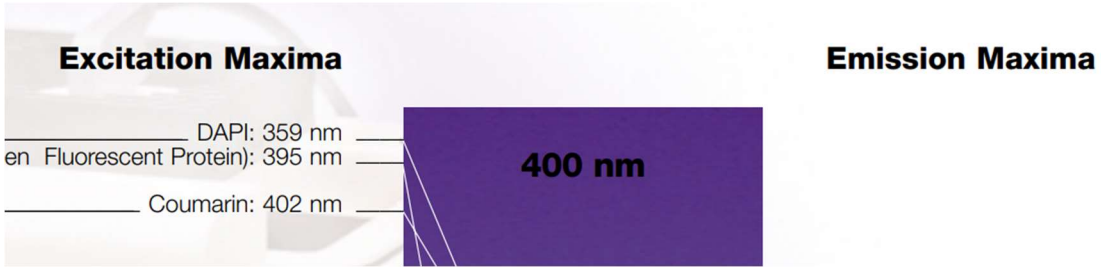
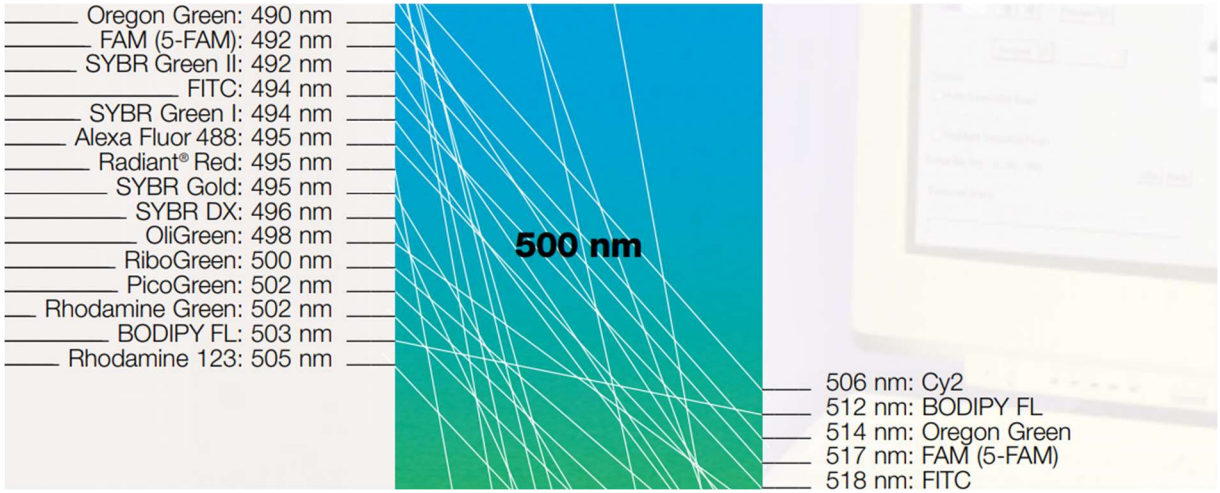
BIO-RAD EX.1009.120

Claims	Exemplary Disclosure of Accused Product
[14.a] the multi-channel detector comprises photodetectors that enable the detection of blue, green, and red fluorescent signals.	The multi-channel detector within QX600 comprises photodetectors that enable the detection of blue, green, and red fluorescent signals, both literally and under the doctrine of equivalents. The QX600's photodetectors are capable of detecting blue, green, and red fluorescent signals. For example, the QX600 detects electromagnetic waves at the emission wavelength of six fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590). *See, e.g.*: Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 On information and belief, the QX600 comprises photodetectors at least capable of detecting

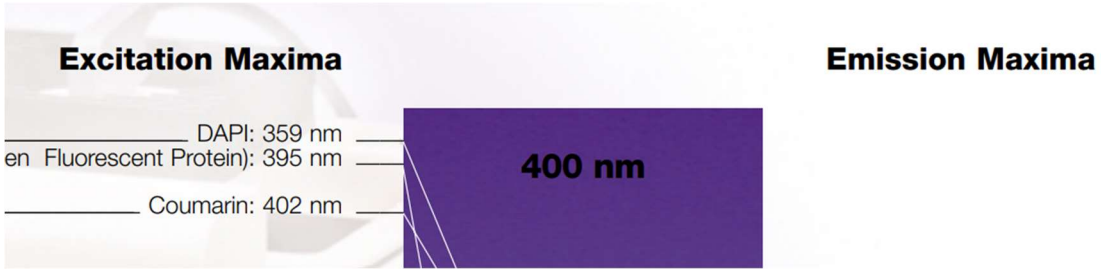
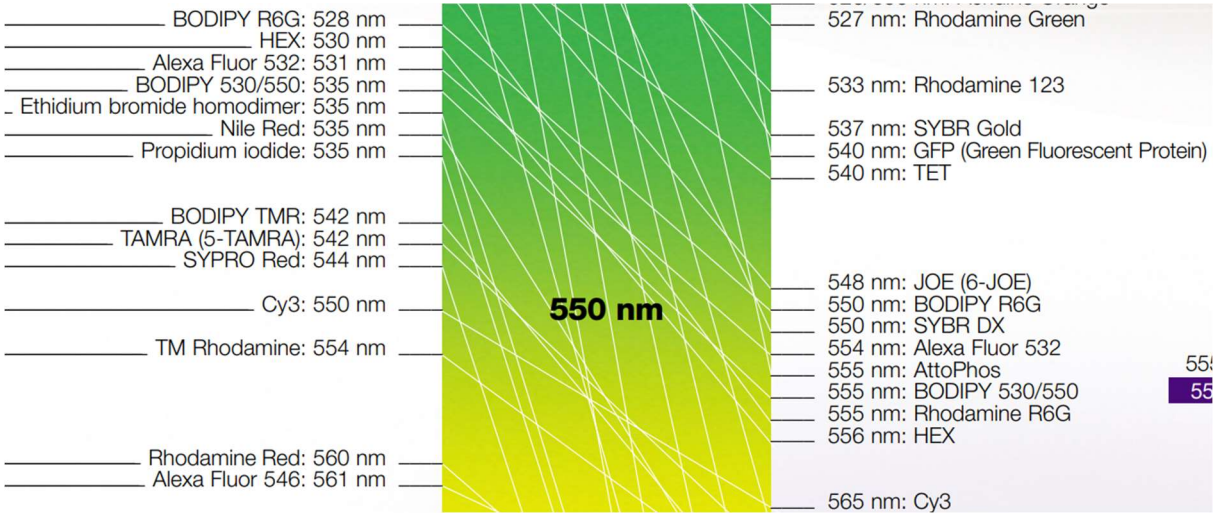
BIO-RAD EX.1009.121

Claims	Exemplary Disclosure of Accused Product
	electromagnetic signals with wavelengths near the emission wavelengths of the six fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590). *See, e.g.* (QX600 Droplet Reader detects using three interrogation points across defined spectrums for each of the six fluorophores): Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 The FAM fluorophore is a green fluorophore. *See, e.g.*:

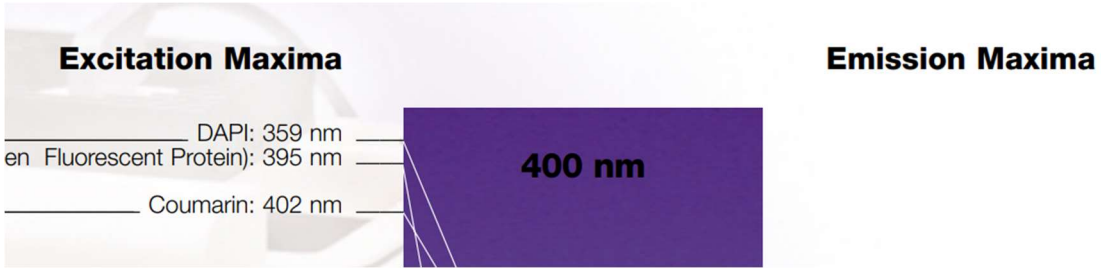
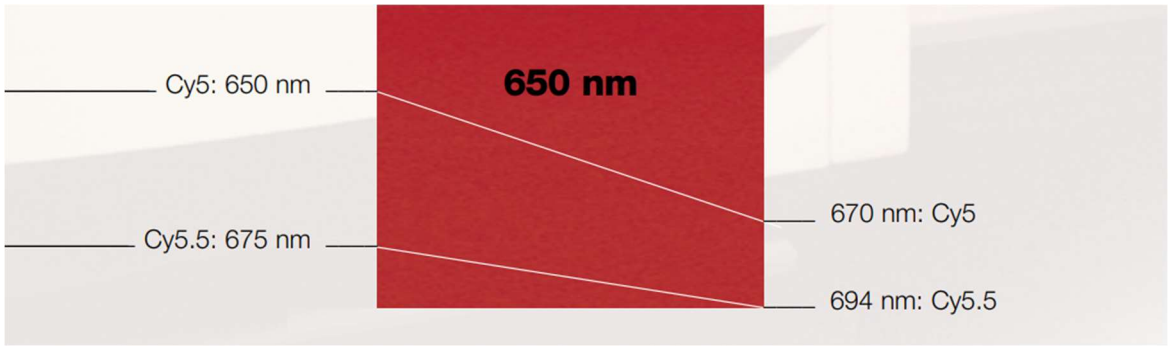
BIO-RAD EX.1009.122

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364  CT0479364 at CT0479364

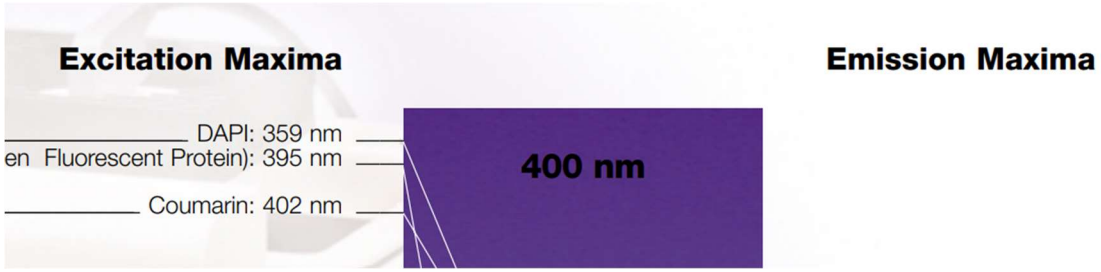
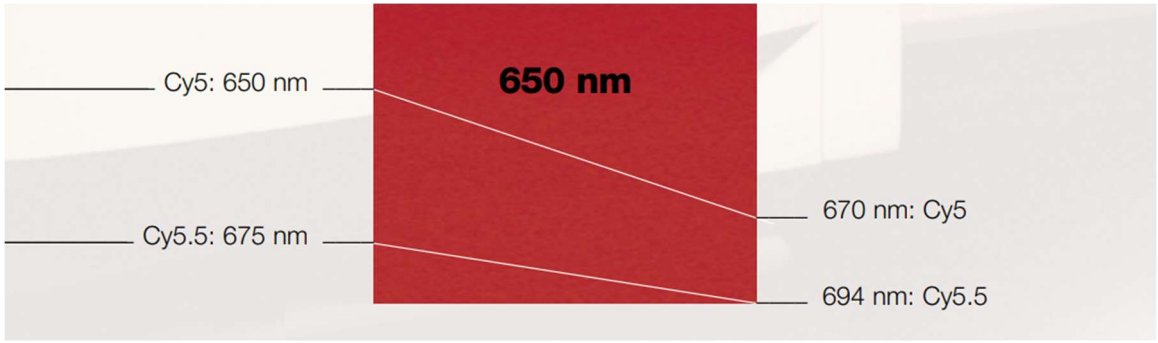
BIO-RAD EX.1009.123

Claims	Exemplary Disclosure of Accused Product
	The HEX fluorophore is a green and yellow fluorophore. *See, e.g.*:  CT0479364 at CT0479364  CT0479364 at CT0479364

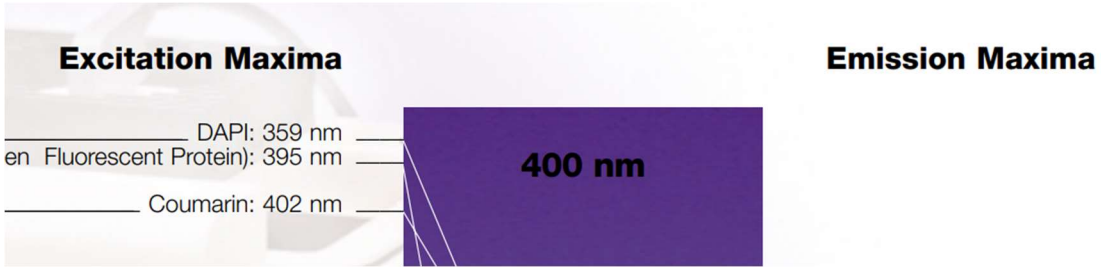
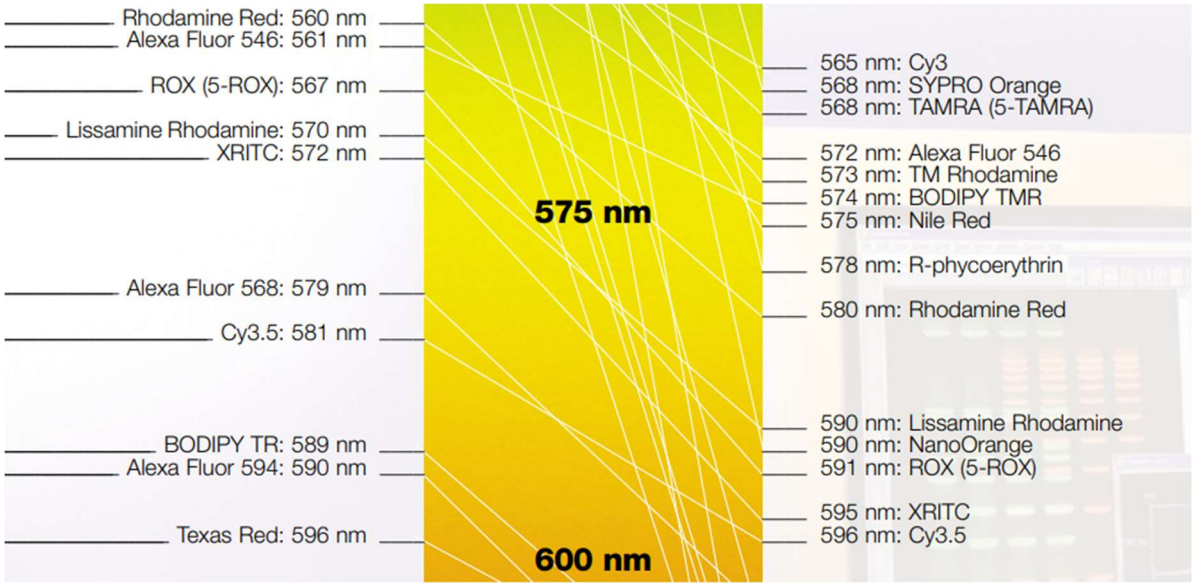
BIO-RAD EX.1009.124

Claims	Exemplary Disclosure of Accused Product
	The Cy5 fluorophore is a red fluorophore. *See, e.g.*:  CT0479364 at CT0479364  CT0479364 at CT0479364

BIO-RAD EX.1009.125

Claims	Exemplary Disclosure of Accused Product
	The Cy5.5 fluorophore is a red fluorophore. *See, e.g.*:  CT0479364 at CT0479364  CT0479364 at CT0479364 The ROX fluorophore is a yellow fluorophore. *See, e.g.*:

BIO-RAD EX.1009.126

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364  CT0479364 at CT0479364

BIO-RAD EX.1009.127

Claims	Exemplary Disclosure of Accused Product
	The ATTO 590 fluorophore has an excitation wavelength of about 593 nm and an emission wavelength of about 622nm. *See, e.g.*:

ATTO-TEC GmbH
Martinshardt 7
D-57074 Siegen
Germany
Phone: +49 271 23853 – 0
FAX: +49 271 23853 – 11
E-mail: info@atto-tec.com
http: www.atto-tec.com



ATTO-TEC

Revised: 2023-11-06

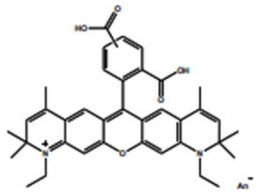
Product Information: ATTO 590

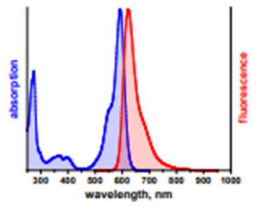


ATTO 590 is a novel fluorescent label belonging to the class of Rhodamine dyes. The dye is designed for application in the area of life science, e.g. labeling of DNA, RNA or proteins. Characteristic features of the label are strong absorption, high fluorescence quantum yield, high thermal and photo-stability, and very little triplet formation. The dye is highly suitable for single-molecule detection applications and high-resolution microscopy.
As supplied **ATTO 590** consists of a mixture of two isomers with practically identical absorption and fluorescence. On request the dye can also be supplied as single isomer.

Optical data of the carboxy derivative *(in PBS, pH 7.4):*

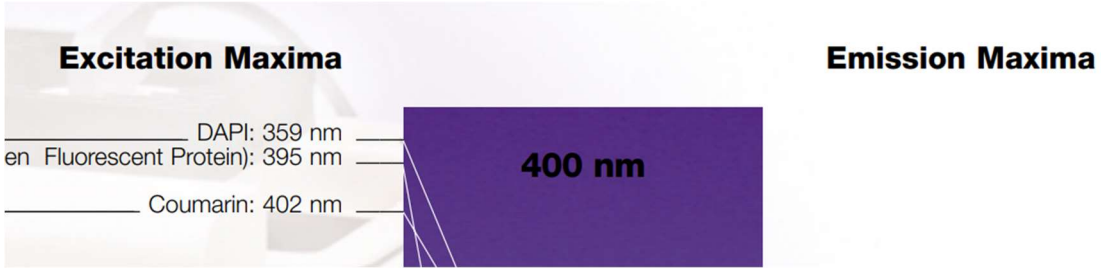
λ_{abs}	=	593 nm
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}
λ_{fl}	=	622 nm
η_{fl}	=	80 %
τ_{fl}	=	3.7 ns
CF_{260}	=	0.39
CF_{280}	=	0.43



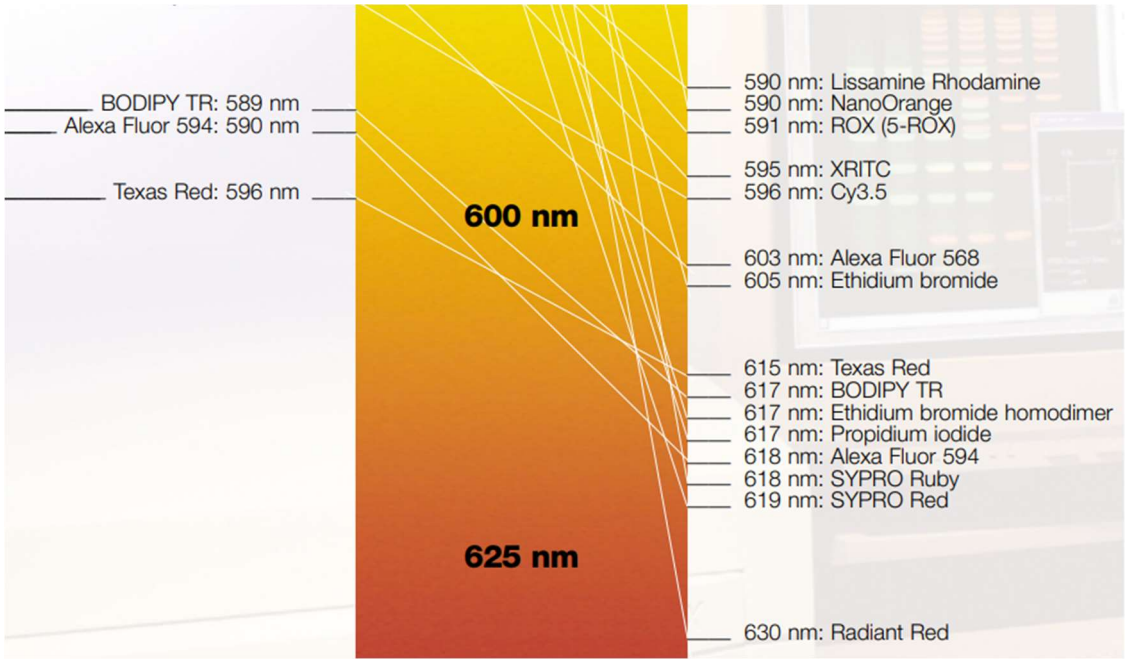


Spectra available in digitized form (excel file) on
http://www.atto-tec.com

BIO-RAD EX.1009.128

Claims	Exemplary Disclosure of Accused Product
	CT0479362 at CT0479362 The ATTO 590 fluorophore's emission wavelength of about 622nm makes ATTO 590 a red fluorophore. *See, e.g.*:  CT0479364 at CT0479364

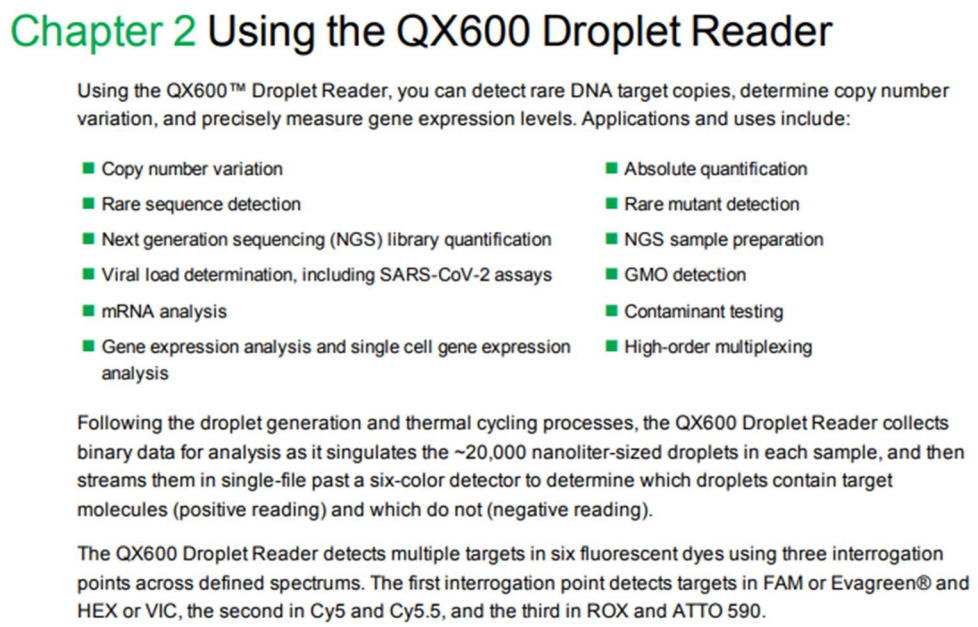
BIO-RAD EX.1009.129

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364 According to at least the examples above, the multi-channel detector within QX600 comprises photodetectors that enable the detection of blue, green (e.g., FAM wavelength), and red (e.g., Cy5, Cy5.5, and ATTO 590 wavelength) fluorescent signals, both literally and under the doctrine of equivalents.

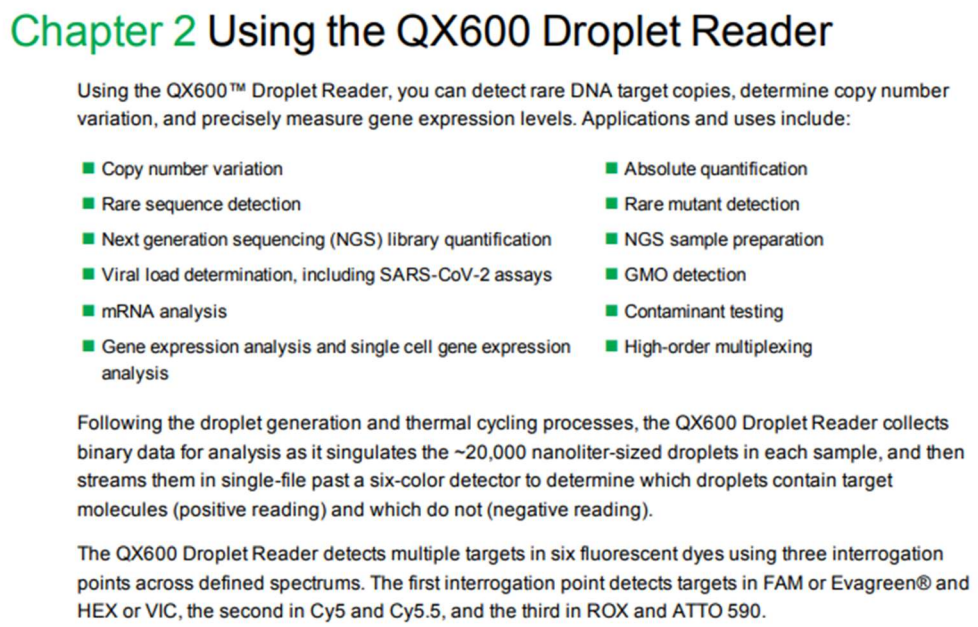
BIO-RAD EX.1009.130

Claims	Exemplary Disclosure of Accused Product
[15.pre] 15. The system of claim 5, wherein	Caltech incorporates the discussion from claim 5, *supra,* in its entirety. As discussed therein, the QX600 is a system comprising each and every element of claim 5, both literally and under the doctrine of equivalents.
[15.a] the multi-channel detector comprises photodetectors that enable the detection ranges selected from the group consisting of from about 483 nm to about 533 nm, from about 523 nm to about 568 nm, from about 558 nm to about 610 nm, from about 615 nm to about 670 nm, and any combinations thereof.	The multi-channel detector within QX600 comprises photodetectors that enable the detection ranges selected from the group consisting of from about 483 nm to about 533 nm, from about 523 nm to about 568 nm, from about 558 nm to about 610 nm, from about 615 nm to about 670 nm, and any combinations thereof, both literally and under the doctrine of equivalents. For example, the QX600 detects electromagnetic waves at the emission wavelength of fluorophores (e.g., FAM, HEX, Cy5, ROX, and ATTO 590). *See, e.g.*:

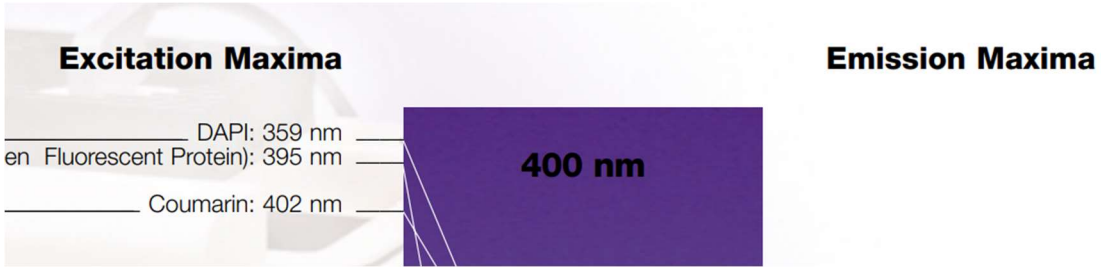
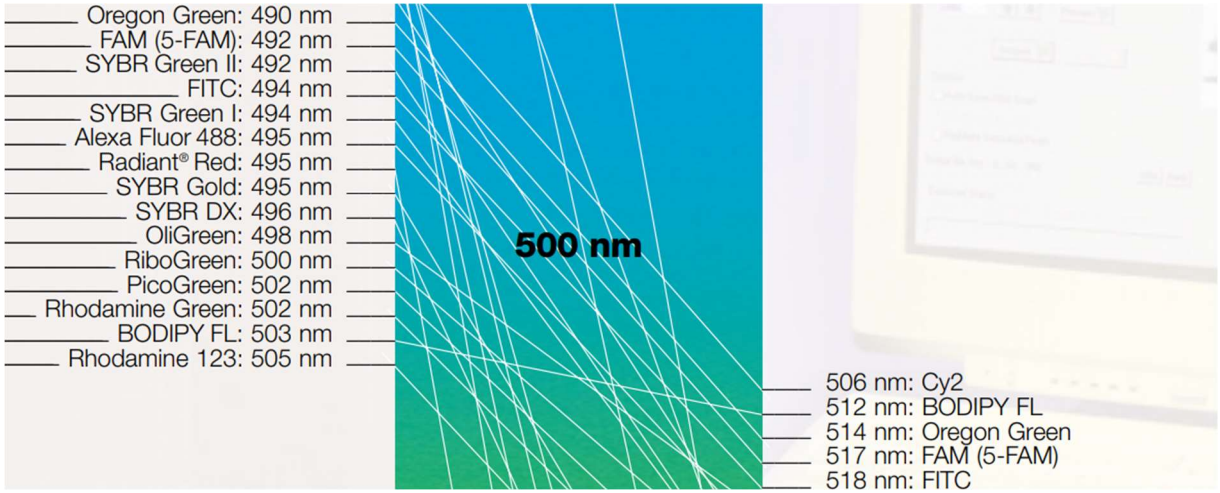
BIO-RAD EX.1009.131

Claims	Exemplary Disclosure of Accused Product
	 Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 On information and belief, the QX600 comprises photodetectors at least capable of detecting electromagnetic signals with wavelengths near the emission wavelengths of the fluorophores (e.g., FAM, HEX, Cy5, ROX, and ATTO 590). *See, e.g.* (QX600 Droplet Reader detects using three interrogation points across defined spectrums for each of the fluorophores):

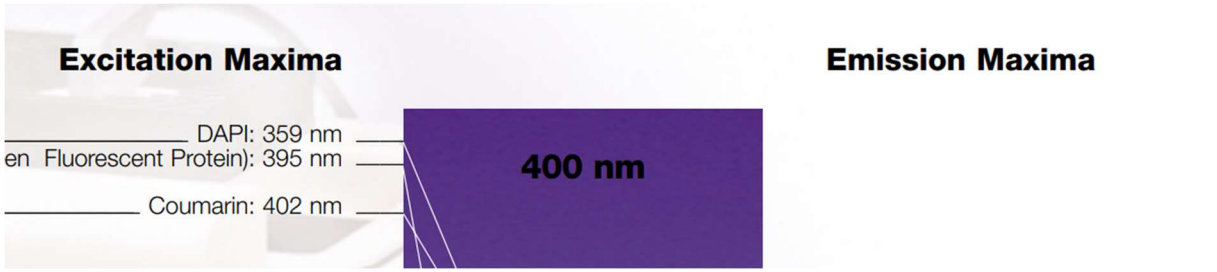
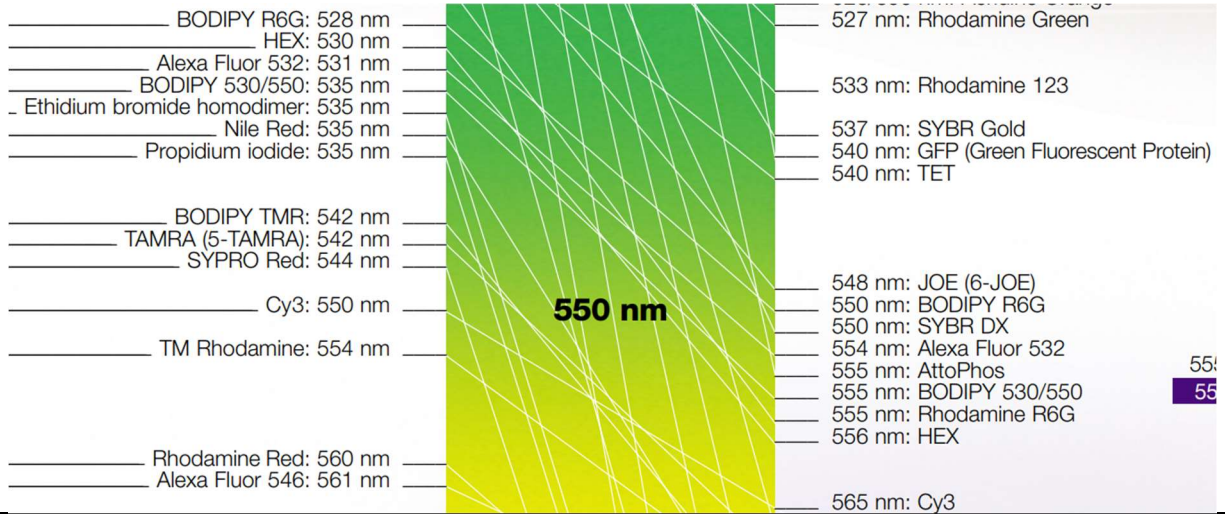
BIO-RAD EX.1009.132

Claims	Exemplary Disclosure of Accused Product
	 CT0479068 at CT0479090 The FAM fluorophore has a maximum emission wavelength of from about 483 nm to about 533 nm (e.g., 517 nm). *See, e.g.*:

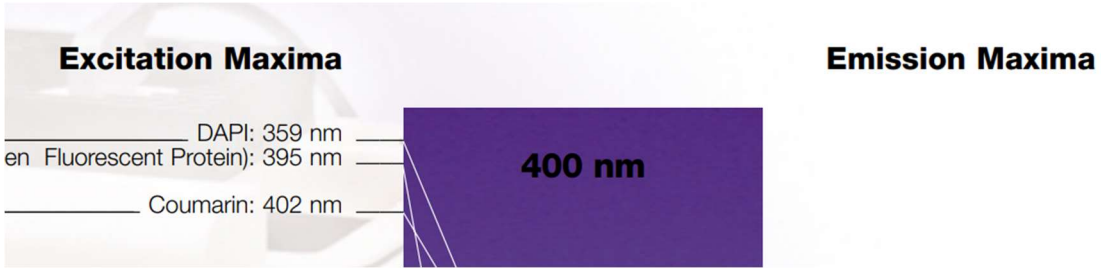
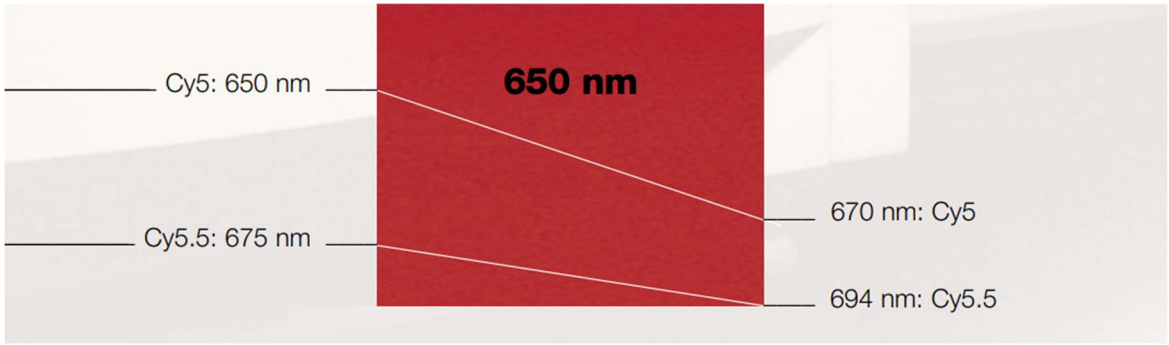
BIO-RAD EX.1009.133

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364  CT0479364 at CT0479364

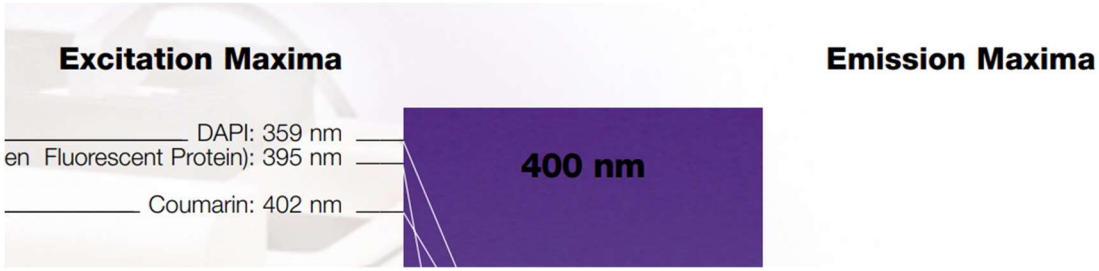
BIO-RAD EX.1009.134

Claims	Exemplary Disclosure of Accused Product
	The HEX fluorophore has a maximum emission wavelength of from about 523 nm to about 568 nm, (e.g., 556 nm). *See, e.g.*:  CT0479364 at CT0479364 

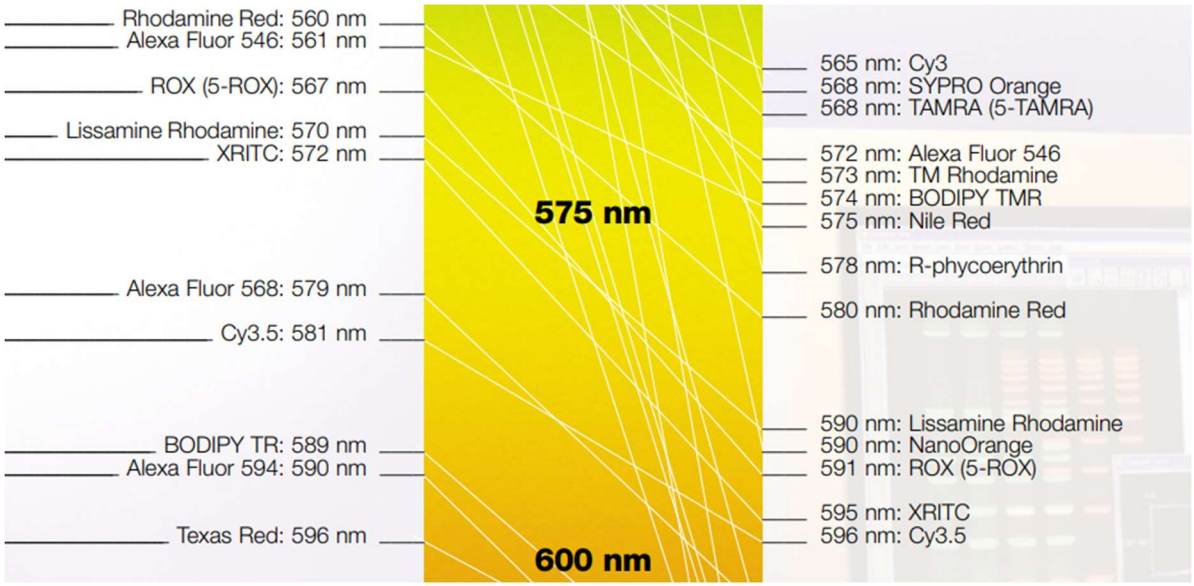
BIO-RAD EX.1009.135

Claims	Exemplary Disclosure of Accused Product
	CT0479364 at CT0479364 The Cy5 fluorophore has a maximum emission wavelength of from about 615 nm to about 670 nm (e.g., 670 nm). *See, e.g.*:  CT0479364 at CT0479364  CT0479364 at CT0479364

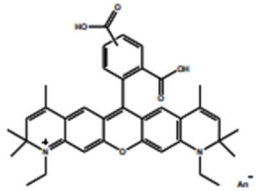
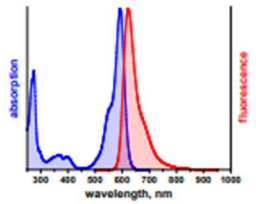
BIO-RAD EX.1009.136

Claims	Exemplary Disclosure of Accused Product
	The ROX fluorophore has a maximum emission wavelength of from about 558 nm to about 610 nm (e.g., 591 nm). *See, e.g.*:  CT0479364 at CT0479364

BIO-RAD EX.1009.137

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364 The ATTO 590 fluorophore has a maximum emission wavelength of from about 615 nm to about 670 nm (e.g., 622 nm). *See, e.g.*:

BIO-RAD EX.1009.138

Claims	Exemplary Disclosure of Accused Product
	ATTO-TEC GmbH Martinshardt 7 D-57074 Siegen Germany *Phone:* +49 271 23853 – 0 *FAX:* +49 271 23853 – 11 *E-mail:* info@atto-tec.com *http:* www.atto-tec.com  ATTO-TEC *Revised:* 2023-11-06 **Product Information: ATTO 590**  **ATTO 590** is a novel fluorescent label belonging to the class of Rhodamine dyes. The dye is designed for application in the area of life science, e.g. labeling of DNA, RNA or proteins. Characteristic features of the label are strong absorption, high fluorescence quantum yield, high thermal and photo-stability, and very little triplet formation. The dye is highly suitable for single-molecule detection applications and high-resolution microscopy. As supplied **ATTO 590** consists of a mixture of two isomers with practically identical absorption and fluorescence. On request the dye can also be supplied as single isomer. ***Optical data of the carboxy derivative*** *(in PBS, pH 7.4):* λ_{abs} = 593 nm ε_{max} = 1.2 x 10^5 M^{-1} cm^{-1} λ_{fl} = 622 nm η_{fl} = 80 % τ_{fl} = 3.7 ns CF_{260} = 0.39 CF_{280} = 0.43   Spectra available in digitized form (excel file) on http://www.atto-tec.com CT0479362 at CT0479362

BIO-RAD EX.1009.139

Claims	Exemplary Disclosure of Accused Product
	The multi-channel detector within QX600 comprises photodetectors that enable the detection ranges selected from the group consisting of from about 483 nm to about 533 nm (e.g., wavelengths near the FAM emission wavelength of 517 nm), from about 523 nm to about 568 nm(e.g., wavelengths near the HEX emission wavelength of 556 nm), from about 558 nm to about 610 nm (e.g., wavelengths near the ROX emission wavelength of 591 nm), from about 615 nm to about 670 nm (e.g., wavelengths near the Cy5 emission wavelength of 670 nm, wavelengths near the ATTO 590 emission wavelength of 622 nm), and any combinations thereof, both literally and under the doctrine of equivalents.
[18.pre] 18. The system of claim 5, further comprising	Caltech incorporates the discussion from claim 5, *supra,* in its entirety. As discussed therein, the QX600 is a system comprising each and every element of claim 5, both literally and under the doctrine of equivalents.
[18.a] a display coupled to the processor controlled analyzer to visualize a plot of the first and second values.	The QX 600 comprises a display coupled to the processor controlled analyzer to visualize a plot of the first and second values, both literally and under the doctrine of equivalents. For example, operation of the QX600 Droplet Reader within the QX600 System involves the operation of additional components (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software). *See, e.g.*:

BIO-RAD EX.1009.140

Claims	Exemplary Disclosure of Accused Product
	## Included Items and Additional Components This section identifies the functional items included with your droplet reader purchase, as well as additional components and consumables. For information on items you may need for a full ddPCR cycle, see Appendix E, Ordering Information. Table 5 explains the components that ship with the QX600 Droplet Reader. **Table 5. QX600 Droplet Reader and Included Components** **Component** / **Description** / **Catalog Number** QX600 Droplet Reader / Droplet reading instrument / 12013328 USB cable and power cord / USB cable that connects the computer to the instrument and power cord that connects the instrument to the power source **Note:** To obtain cables or power cords, contact Bio-Rad technical support. / Included Droplet reader plate holders (2) / Positions and secures the 96-well plate in the droplet reader plate compartment / 12006834 To ensure you have everything you need to complete runs on the QX600 Droplet Reader and analyze your results, Table 6 identifies additional components and accessories that you can purchase separately. **Table 6. Additional Accessories and Consumables** **Item** / **Description** / **Catalog Number** Computer / Computer that connects to the QX600 Droplet Reader for data collection and analysis / 12017458 96-well PCR plates / ddPCR 96-well plates / 12001925 Rainin pipets / 20 µl for sample loading 50 µl for droplet transfer 8-channel, 200 µl for oil / L-20, L8-20 L-50, L8-50 L8-200 CT0479068 at CT0479095

BIO-RAD EX.1009.141

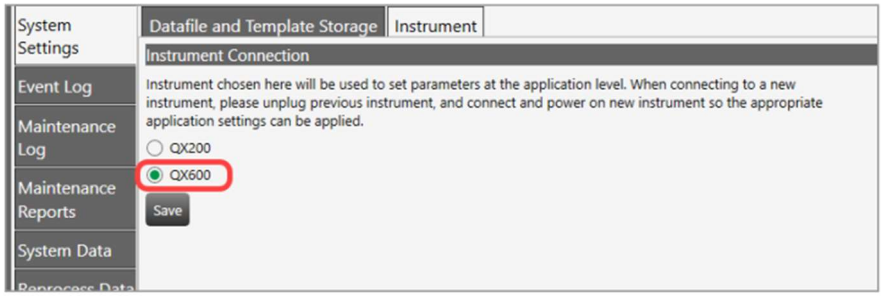
Claims	Exemplary Disclosure of Accused Product
	# Appendix E Ordering Information This appendix contains descriptions and catalog numbers for new or replacement instruments, accessories, and consumables for Bio-Rad's ddPCR products, including your QX600 Droplet Reader. **ddPCR System and Instrument Packages** Table 41 contains information on Bio-Rad Laboratories, Inc. Droplet Digital PCR systems, as well as individual ddPCR instruments and included accessories. **Table 41. ddPCR System and Instrument Packages** | Product	Description	Catalog Number
---	---	---
QX200 Droplet Digital PCR System	Includes the QX200 Droplet Generator, QX200 Droplet Reader, computer, software, and consumables	1864001
QX600 Droplet Digital PCR System	Includes the QX200 Droplet Generator, QX600 Droplet Reader, computer, software, and consumables	17007769
QX600 AutoDG™ Droplet Digital PCR System	Includes the Automated Droplet Generator (AutoDG), QX600 Droplet Reader, computer, software, and consumables	17008371
QX200 Droplet Generator	Includes the droplet generator (1), cartridges and gaskets (24 each), cartridge holders (2), and power cord (1)	1864002
QX200 Droplet Reader	Includes the droplet reader (1), plate holders (2), USB cable (1), and power cord (1)	1864003
QX600 Droplet Reader	Includes the droplet reader (1), plate holders (2), USB cable (1), and power cord (1)	12013328
Automated Droplet Generator	Includes the automated droplet generator (1), cooling block accessory (1), oil purge reservoir (1), and power cord (1)	1864101
C1000 Thermal Cycler	Thermal cycler with 384-well reaction module	1851384	 CT0479068 at CT0479276

BIO-RAD EX.1009.142

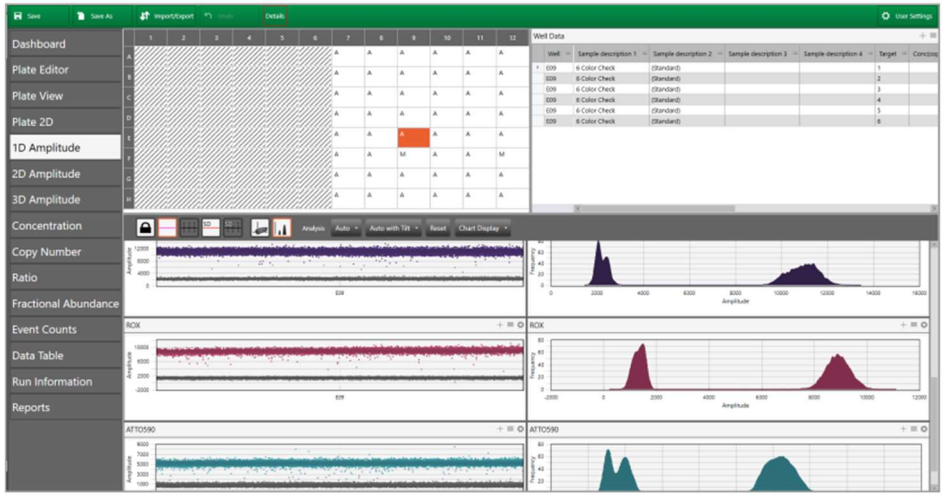
Claims	Exemplary Disclosure of Accused Product
	QX600 Droplet Reader Accessories Table 42 contains information on accessories for the QX600 Droplet Reader. **Table 43. QX600 Droplet Reader Accessories** CT0479068 at CT0479278 QX600's QX Manager Software is run on the attached computer to display the collected fluorophore signal data and conduct analysis on the same. *See, e.g.*:

Product	Description	Catalog Number
Computer	Computer that connects to the QX600 Droplet Reader for data collection and analysis	12017458
USB cable and power cord	Cable connecting the computer to the instrument and power cord connecting the instrument to the power source **Note:** Contact Bio-Rad Technical Support for replacement information.	Included
Droplet reader plate holders (2)	Used to position the 96-well plate in the droplet reader plate compartment	12006834

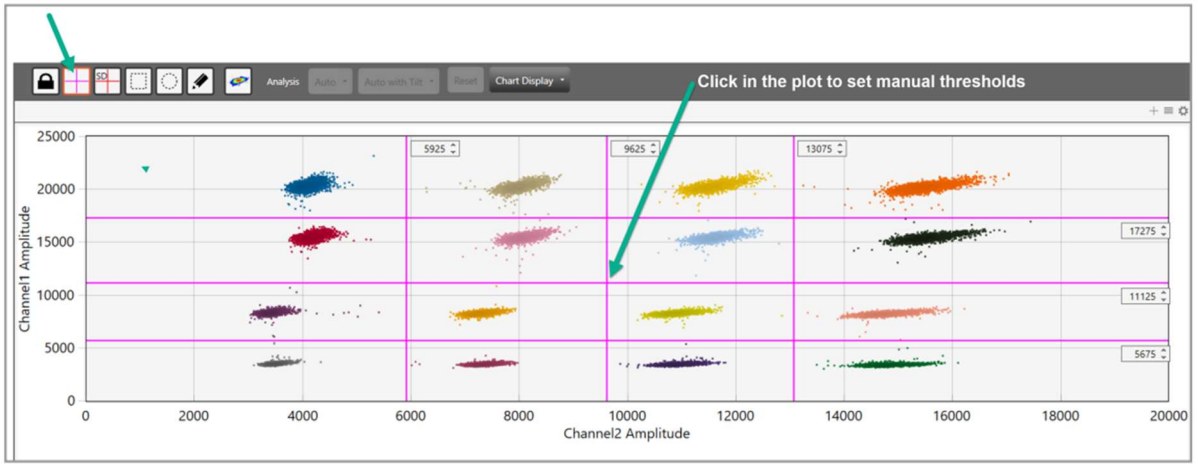
BIO-RAD EX.1009.143

Claims	Exemplary Disclosure of Accused Product
	Selecting the Instrument After First Sign-In When you open the software and sign in for the first time, the instrument connection window appears and you must select the instrument that is connected to the computer. This procedure assumes the instrument is connected to the computer and QX Manager Software is installed. You can use QX Manager Software with the QX200 Droplet Reader and the QX600 Droplet Reader. **To select the instrument** ▶ Select QX600, and then click Save.  **Important:** If your laboratory uses both instruments, you can change instruments at any time. See Changing Instruments on page 165 for information. CT0479068 at CT0479105 The QX Manager Software provides visual plots of first values (e.g., channel intensity values) and second values (e.g., channel wavelength values). *See e.g.*:

BIO-RAD EX.1009.144

Claims	Exemplary Disclosure of Accused Product
	1D Amplitude Use the 1D Amplitude analysis window to view droplet amplitudes for each channel, in selection order, for each well selected in the Well Selector. The following graphic shows six colors in standard-size droplets.  CT0479068 at CT0479186

BIO-RAD EX.1009.145

Claims	Exemplary Disclosure of Accused Product
	 CT0479068 at CT0479252 According to at least the examples above, the QX600 comprises a display coupled to the processor controlled analyzer to visualize a plot of the first (e.g., channel intensity) and second (e.g., channel wavelength) values, both literally and under the doctrine of equivalents.

BIO-RAD EX.1009.146

Claims	Exemplary Disclosure of Accused Product
[19.pre] 19. A system comprising:	To the extent the preamble is found to be limiting, the QX600 is a system comprising each and every element below, both literally and under the doctrine of equivalents. For example, the QX600 is a "system" comprising multiple components, each of which is itself a "system," including at least a droplet generator (QX200 Droplet Generator or AutoDG), QX600 Droplet Reader, thermal cycler, and a computer, as well as software and consumables. *See, e.g.*:

BIO-RAD EX.1009.147

Claims	Exemplary Disclosure of Accused Product
	# Appendix E Ordering Information This appendix contains descriptions and catalog numbers for new or replacement instruments, accessories, and consumables for Bio-Rad's ddPCR products, including your QX600 Droplet Reader. **ddPCR System and Instrument Packages** Table 41 contains information on Bio-Rad Laboratories, Inc. Droplet Digital PCR systems, as well as individual ddPCR instruments and included accessories. **Table 41. ddPCR System and Instrument Packages** | Product	Description	Catalog Number
---	---	---
QX200 Droplet Digital PCR System	Includes the QX200 Droplet Generator, QX200 Droplet Reader, computer, software, and consumables	1864001
QX600 Droplet Digital PCR System	Includes the QX200 Droplet Generator, QX600 Droplet Reader, computer, software, and consumables	17007769
QX600 AutoDG™ Droplet Digital PCR System	Includes the Automated Droplet Generator (AutoDG), QX600 Droplet Reader, computer, software, and consumables	17008371
QX200 Droplet Generator	Includes the droplet generator (1), cartridges and gaskets (24 each), cartridge holders (2), and power cord (1)	1864002
QX200 Droplet Reader	Includes the droplet reader (1), plate holders (2), USB cable (1), and power cord (1)	1864003
QX600 Droplet Reader	Includes the droplet reader (1), plate holders (2), USB cable (1), and power cord (1)	12013328
Automated Droplet Generator	Includes the automated droplet generator (1), cooling block accessory (1), oil purge reservoir (1), and power cord (1)	1864101
C1000 Thermal Cycler	Thermal cycler with 384-well reaction module	1851384	 CT0479068 at CT0479276

BIO-RAD EX.1009.148

Claims	Exemplary Disclosure of Accused Product
	QX600 Droplet Reader Accessories Table 42 contains information on accessories for the QX600 Droplet Reader. **Table 43. QX600 Droplet Reader Accessories** **Product** / **Description** / **Catalog Number** Computer / Computer that connects to the QX600 Droplet Reader for data collection and analysis / 12017458 USB cable and power cord / Cable connecting the computer to the instrument and power cord connecting the instrument to the power source **Note:** Contact Bio-Rad Technical Support for replacement information. / Included Droplet reader plate holders (2) / Used to position the 96-well plate in the droplet reader plate compartment / 12006834 CT0479068 at CT0479278 According to at least the examples provided above, and as explained in further detail for each and every element below, the QX600 is a system comprising each and every element of independent claim 19, both literally and under the doctrine of equivalents.

BIO-RAD EX.1009.149

Claims	Exemplary Disclosure of Accused Product
[19.a] a processor;	The QX600 comprises a processor, both literally and under the doctrine of equivalents. For example, operation of the QX600 Droplet Reader involves the operation of additional components (e.g., a computer that includes a processor, connected to the QX600 Droplet Reader for data collection and analysis). *See, e.g.*:

BIO-RAD EX.1009.150

Claims	Exemplary Disclosure of Accused Product
	## Included Items and Additional Components This section identifies the functional items included with your droplet reader purchase, as well as additional components and consumables. For information on items you may need for a full ddPCR cycle, see Appendix E, Ordering Information. Table 5 explains the components that ship with the QX600 Droplet Reader. **Table 5. QX600 Droplet Reader and Included Components** **Component** / **Description** / **Catalog Number** QX600 Droplet Reader / Droplet reading instrument / 12013328 USB cable and power cord / USB cable that connects the computer to the instrument and power cord that connects the instrument to the power source **Note:** To obtain cables or power cords, contact Bio-Rad technical support. / Included Droplet reader plate holders (2) / Positions and secures the 96-well plate in the droplet reader plate compartment / 12006834 To ensure you have everything you need to complete runs on the QX600 Droplet Reader and analyze your results, Table 6 identifies additional components and accessories that you can purchase separately. **Table 6. Additional Accessories and Consumables** **Item** / **Description** / **Catalog Number** Computer / Computer that connects to the QX600 Droplet Reader for data collection and analysis / 12017458 96-well PCR plates / ddPCR 96-well plates / 12001925 Rainin pipets / 20 µl for sample loading 50 µl for droplet transfer 8-channel, 200 µl for oil / L-20, L8-20 L-50, L8-50 L8-200 CT0479068 at CT0479095

BIO-RAD EX.1009.151

Claims	Exemplary Disclosure of Accused Product
	The processor in the computer connected to the QX600 Droplet Reader is used to run the QX Manager Software. *See, e.g.*: **Connecting and Starting the Instrument** To execute runs on the QX600 Droplet Reader, you must connect the instrument to a computer on which the QX Manager Software is installed and configured. Bio-Rad recommends purchasing at least one configured computer that can remain connected to the QX600 Droplet Reader and be used for adding and running plates in QX Manager Software. For information on additional items you can purchase for your instrument, see Included Items and Additional Components on page 26. **To connect and start the instrument** 1. Connect the USB cable provided by Bio-Rad to the USB port on the back of the QX600 Droplet Reader, and then to the computer. 2. Press the Power button on the instrument. The Power indicator light turns solid green to indicate that power is on. 3. Turn on the connected computer and log in with a Windows account. For information, see your system administrator. 4. Open and log into QX Manager Software. **Important:** The first time you log into the software, the Instrument window appears and you must select the instrument you are using. For information on selecting the correct instrument in QX Manager Software, see Selecting the Instrument After First Sign-In on page 36. CT0479068 at CT0479097

BIO-RAD EX.1009.152

Claims	Exemplary Disclosure of Accused Product
	The computer connected to the QX600 Droplet Reader was included in sales of the QX600 or convoyed in sales of the QX600 Droplet Reader. *See, e.g.*:

Appendix E Ordering Information

This appendix contains descriptions and catalog numbers for new or replacement instruments, accessories, and consumables for Bio-Rad's ddPCR products, including your QX600 Droplet Reader.

ddPCR System and Instrument Packages

Table 41 contains information on Bio-Rad Laboratories, Inc. Droplet Digital PCR systems, as well as individual ddPCR instruments and included accessories.

Table 41. ddPCR System and Instrument Packages

Product	Description	Catalog Number
QX200 Droplet Digital PCR System	Includes the QX200 Droplet Generator, QX200 Droplet Reader, computer, software, and consumables	1864001
QX600 Droplet Digital PCR System	Includes the QX200 Droplet Generator, QX600 Droplet Reader, computer, software, and consumables	17007769
QX600 AutoDG™ Droplet Digital PCR System	Includes the Automated Droplet Generator (AutoDG), QX600 Droplet Reader, computer, software, and consumables	17008371
QX200 Droplet Generator	Includes the droplet generator (1), cartridges and gaskets (24 each), cartridge holders (2), and power cord (1)	1864002
QX200 Droplet Reader	Includes the droplet reader (1), plate holders (2), USB cable (1), and power cord (1)	1864003
QX600 Droplet Reader	Includes the droplet reader (1), plate holders (2), USB cable (1), and power cord (1)	12013328
Automated Droplet Generator	Includes the automated droplet generator (1), cooling block accessory (1), oil purge reservoir (1), and power cord (1)	1864101
C1000 Thermal Cycler	Thermal cycler with 384-well reaction module	1851384

CT0479068 at CT0479276

BIO-RAD EX.1009.153

Claims	Exemplary Disclosure of Accused Product
	QX600 Droplet Reader Accessories Table 42 contains information on accessories for the QX600 Droplet Reader. **Table 43. QX600 Droplet Reader Accessories** **Product** / **Description** / **Catalog Number** Computer / Computer that connects to the QX600 Droplet Reader for data collection and analysis / 12017458 USB cable and power cord / Cable connecting the computer to the instrument and power cord connecting the instrument to the power source **Note:** Contact Bio-Rad Technical Support for replacement information. / Included Droplet reader plate holders (2) / Used to position the 96-well plate in the droplet reader plate compartment / 12006834 CT0479068 at CT0479278 Customers were also instructed to purchase a computer to the extent it was not included in their purchase of the QX600. *See e.g.* ("To ensure you have everything you need to complete runs on the QX600 Droplet reader and analyze your results, Table 6 [listing a computer] identifies additional components and accessories that you can purchase separately."):

BIO-RAD EX.1009.154

Claims	Exemplary Disclosure of Accused Product
	To ensure you have everything you need to complete runs on the QX600 Droplet Reader and analyze your results, Table 6 identifies additional components and accessories that you can purchase separately. **Table 6. Additional Accessories and Consumables** **Item** / **Description** / **Catalog Number** Computer / Computer that connects to the QX600 Droplet Reader for data collection and analysis / 12017458 96-well PCR plates / ddPCR 96-well plates / 12001925 Rainin pipets / 20 µl for sample loading 50 µl for droplet transfer 8-channel, 200 µl for oil / L-20, L8-20 L-50, L8-50 L8-200 CT0479068 at CT0479095 To the extent such computer for connection to the QX600 Droplet Reader was included in sales of the QX600, or in related convoy sales, and/or because Bio-Rad induced and/or contributed to such purchase of such computer for connection to the QX600 Droplet Reader was induced, the QX600 infringes claim 19 directly or indirectly, at least because the QX600 comprises a processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software) both literally and under the doctrine of equivalents. According to at least the examples above, the QX600 Droplet Digital PCR System comprises a processor (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager

BIO-RAD EX.1009.155

Claims	Exemplary Disclosure of Accused Product
	Software), both literally and under the doctrine of equivalents.
[19.b] a display; and	The QX 600 comprises a display, both literally and under the doctrine of equivalents. For example, operation of the QX600 Droplet Reader involves the operation of additional components (e.g., a computer with a display, connected to the QX600 Droplet Reader for data collection and analysis). *See, e.g.*:

BIO-RAD EX.1009.156

Claims	Exemplary Disclosure of Accused Product
	## Included Items and Additional Components This section identifies the functional items included with your droplet reader purchase, as well as additional components and consumables. For information on items you may need for a full ddPCR cycle, see Appendix E, Ordering Information. Table 5 explains the components that ship with the QX600 Droplet Reader. **Table 5. QX600 Droplet Reader and Included Components** **Component** / **Description** / **Catalog Number** QX600 Droplet Reader / Droplet reading instrument / 12013328 USB cable and power cord / USB cable that connects the computer to the instrument and power cord that connects the instrument to the power source **Note:** To obtain cables or power cords, contact Bio-Rad technical support. / Included Droplet reader plate holders (2) / Positions and secures the 96-well plate in the droplet reader plate compartment / 12006834 To ensure you have everything you need to complete runs on the QX600 Droplet Reader and analyze your results, Table 6 identifies additional components and accessories that you can purchase separately. **Table 6. Additional Accessories and Consumables** **Item** / **Description** / **Catalog Number** Computer / Computer that connects to the QX600 Droplet Reader for data collection and analysis / 12017458 96-well PCR plates / ddPCR 96-well plates / 12001925 Rainin pipets / 20 µl for sample loading 50 µl for droplet transfer 8-channel, 200 µl for oil / L-20, L8-20 L-50, L8-50 L8-200 CT0479068 at CT0479095

BIO-RAD EX.1009.157

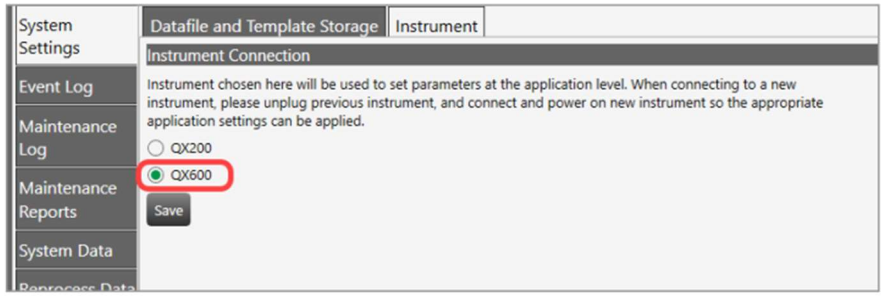
Claims	Exemplary Disclosure of Accused Product
	# Appendix E Ordering Information This appendix contains descriptions and catalog numbers for new or replacement instruments, accessories, and consumables for Bio-Rad's ddPCR products, including your QX600 Droplet Reader. **ddPCR System and Instrument Packages** Table 41 contains information on Bio-Rad Laboratories, Inc. Droplet Digital PCR systems, as well as individual ddPCR instruments and included accessories. **Table 41. ddPCR System and Instrument Packages** | Product	Description	Catalog Number | |---|---|---| | QX200 Droplet Digital PCR System | Includes the QX200 Droplet Generator, QX200 Droplet Reader, computer, software, and consumables | 1864001 | | QX600 Droplet Digital PCR System | Includes the QX200 Droplet Generator, QX600 Droplet Reader, computer, software, and consumables | 17007769 | | QX600 AutoDG™ Droplet Digital PCR System | Includes the Automated Droplet Generator (AutoDG), QX600 Droplet Reader, computer, software, and consumables | 17008371 | | QX200 Droplet Generator | Includes the droplet generator (1), cartridges and gaskets (24 each), cartridge holders (2), and power cord (1) | 1864002 | | QX200 Droplet Reader | Includes the droplet reader (1), plate holders (2), USB cable (1), and power cord (1) | 1864003 | | QX600 Droplet Reader | Includes the droplet reader (1), plate holders (2), USB cable (1), and power cord (1) | 12013328 | | Automated Droplet Generator | Includes the automated droplet generator (1), cooling block accessory (1), oil purge reservoir (1), and power cord (1) | 1864101 | | C1000 Thermal Cycler | Thermal cycler with 384-well reaction module | 1851384 | CT0479068 at CT0479276

BIO-RAD EX.1009.158

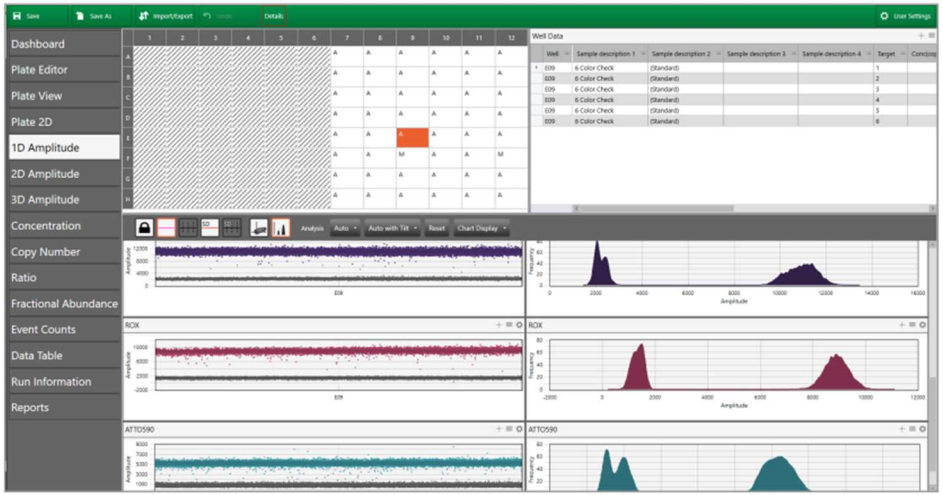
Claims	Exemplary Disclosure of Accused Product
	QX600 Droplet Reader Accessories Table 42 contains information on accessories for the QX600 Droplet Reader. **Table 43. QX600 Droplet Reader Accessories** CT0479068 at CT0479278 QX600's QX Manager Software is run on the attached computer to display the collected fluorophore signal data and conduct analysis on the same. *See, e.g.*:

Product	Description	Catalog Number
Computer	Computer that connects to the QX600 Droplet Reader for data collection and analysis	12017458
USB cable and power cord	Cable connecting the computer to the instrument and power cord connecting the instrument to the power source **Note:** Contact Bio-Rad Technical Support for replacement information.	Included
Droplet reader plate holders (2)	Used to position the 96-well plate in the droplet reader plate compartment	12006834

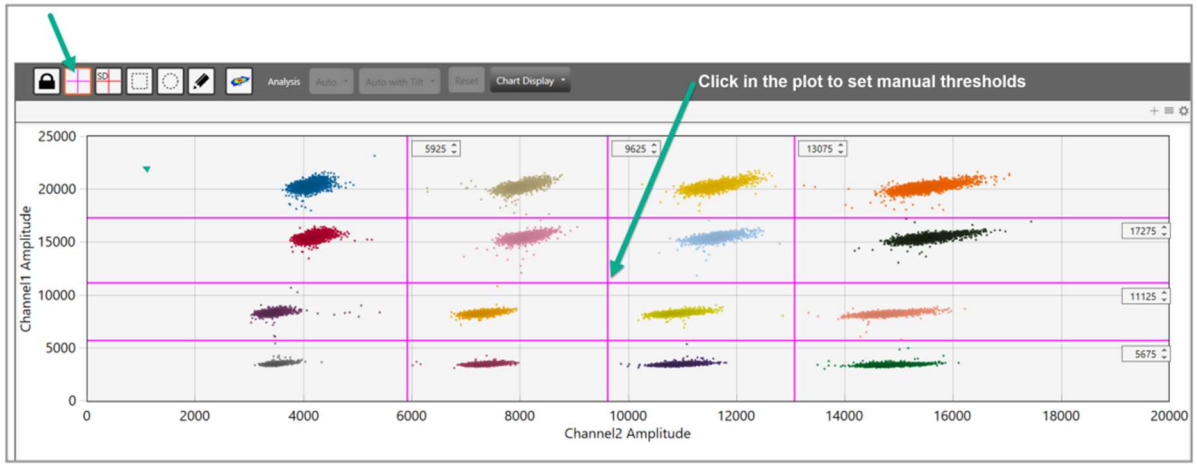
BIO-RAD EX.1009.159

Claims	Exemplary Disclosure of Accused Product
	Selecting the Instrument After First Sign-In When you open the software and sign in for the first time, the instrument connection window appears and you must select the instrument that is connected to the computer. This procedure assumes the instrument is connected to the computer and QX Manager Software is installed. You can use QX Manager Software with the QX200 Droplet Reader and the QX600 Droplet Reader. **To select the instrument** ▶ Select QX600, and then click Save.  **Important:** If your laboratory uses both instruments, you can change instruments at any time. See Changing Instruments on page 165 for information. CT0479068 at CT0479105 The QX Manager Software provides visual plots of first values (e.g., channel intensity values) and second values (e.g., channel wavelength values). *See e.g.*:

BIO-RAD EX.1009.160

Claims	Exemplary Disclosure of Accused Product
	1D Amplitude Use the 1D Amplitude analysis window to view droplet amplitudes for each channel, in selection order, for each well selected in the Well Selector. The following graphic shows six colors in standard-size droplets.  CT0479068 at CT0479186

BIO-RAD EX.1009.161

Claims	Exemplary Disclosure of Accused Product
	 CT0479068 at CT0479252 According to at least the examples above, the QX600 comprises a display, both literally and under the doctrine of equivalents.
[19.c] a non-transitory computer readable medium storing instructions thereon that, when executed by the processor, cause the	The QX 600 comprises a non-transitory computer readable medium storing instructions thereon, both literally and under the doctrine of equivalents. For example, operation of the QX600 Droplet Reader involves the operation of additional components (e.g., a computer with memory and computer code, connected to the QX600 Droplet Reader, to cause the computer's processor to execute operations, for data collection and analysis). *See, e.g.*:

BIO-RAD EX.1009.162

Claims	Exemplary Disclosure of Accused Product
processor to:	

Included Items and Additional Components

This section identifies the functional items included with your droplet reader purchase, as well as additional components and consumables. For information on items you may need for a full ddPCR cycle, see Appendix E, Ordering Information.

Table 5 explains the components that ship with the QX600 Droplet Reader.

Table 5. QX600 Droplet Reader and Included Components

Component	Description	Catalog Number
QX600 Droplet Reader	Droplet reading instrument	12013328
USB cable and power cord	USB cable that connects the computer to the instrument and power cord that connects the instrument to the power source **Note:** To obtain cables or power cords, contact Bio-Rad technical support.	Included
Droplet reader plate holders (2)	Positions and secures the 96-well plate in the droplet reader plate compartment	12006834

To ensure you have everything you need to complete runs on the QX600 Droplet Reader and analyze your results, Table 6 identifies additional components and accessories that you can purchase separately.

Table 6. Additional Accessories and Consumables

Item	Description	Catalog Number
Computer	Computer that connects to the QX600 Droplet Reader for data collection and analysis	12017458
96-well PCR plates	ddPCR 96-well plates	12001925
Rainin pipets	20 µl for sample loading 50 µl for droplet transfer 8-channel, 200 µl for oil	L-20, L8-20 L-50, L8-50 L8-200

BIO-RAD EX.1009.163

Claims	Exemplary Disclosure of Accused Product
	CT0479068 at CT0479095

Appendix E Ordering Information

This appendix contains descriptions and catalog numbers for new or replacement instruments, accessories, and consumables for Bio-Rad's ddPCR products, including your QX600 Droplet Reader.

ddPCR System and Instrument Packages

Table 41 contains information on Bio-Rad Laboratories, Inc. Droplet Digital PCR systems, as well as individual ddPCR instruments and included accessories.

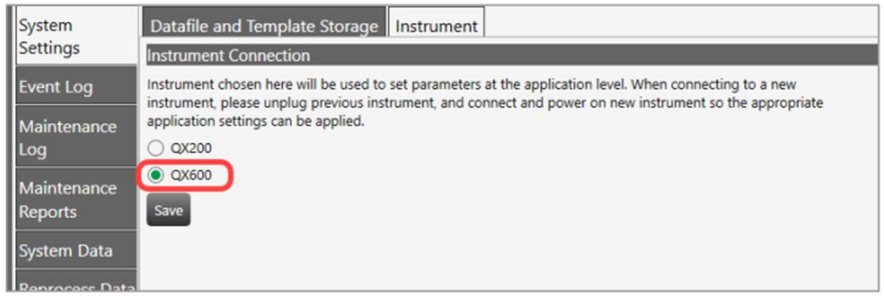
Table 41. ddPCR System and Instrument Packages

Product	Description	Catalog Number
QX200 Droplet Digital PCR System	Includes the QX200 Droplet Generator, QX200 Droplet Reader, computer, software, and consumables	1864001
QX600 Droplet Digital PCR System	Includes the QX200 Droplet Generator, QX600 Droplet Reader, computer, software, and consumables	17007769
QX600 AutoDG™ Droplet Digital PCR System	Includes the Automated Droplet Generator (AutoDG), QX600 Droplet Reader, computer, software, and consumables	17008371
QX200 Droplet Generator	Includes the droplet generator (1), cartridges and gaskets (24 each), cartridge holders (2), and power cord (1)	1864002
QX200 Droplet Reader	Includes the droplet reader (1), plate holders (2), USB cable (1), and power cord (1)	1864003
QX600 Droplet Reader	Includes the droplet reader (1), plate holders (2), USB cable (1), and power cord (1)	12013328
Automated Droplet Generator	Includes the automated droplet generator (1), cooling block accessory (1), oil purge reservoir (1), and power cord (1)	1864101
C1000 Thermal Cycler	Thermal cycler with 384-well reaction module	1851384

BIO-RAD EX.1009.164

Claims	Exemplary Disclosure of Accused Product
	CT0479068 at CT0479276 **QX600 Droplet Reader Accessories** Table 42 contains information on accessories for the QX600 Droplet Reader. **Table 43. QX600 Droplet Reader Accessories** **Product** / **Description** / **Catalog Number** Computer / Computer that connects to the QX600 Droplet Reader for data collection and analysis / 12017458 USB cable and power cord / Cable connecting the computer to the instrument and power cord connecting the instrument to the power source **Note:** Contact Bio-Rad Technical Support for replacement information. / Included Droplet reader plate holders (2) / Used to position the 96-well plate in the droplet reader plate compartment / 12006834 CT0479068 at CT0479278 On information and belief, the attached computer comprises a non-transitory computer readable medium storing instructions thereon, at least for the purposes of storing the QX Manager Software and collected fluorophore signal data for analysis. *See, e.g.*:

BIO-RAD EX.1009.165

Claims	Exemplary Disclosure of Accused Product
	Selecting the Instrument After First Sign-In When you open the software and sign in for the first time, the instrument connection window appears and you must select the instrument that is connected to the computer. This procedure assumes the instrument is connected to the computer and QX Manager Software is installed. You can use QX Manager Software with the QX200 Droplet Reader and the QX600 Droplet Reader. **To select the instrument** ▶ Select QX600, and then click Save.  **Important:** If your laboratory uses both instruments, you can change instruments at any time. See Changing Instruments on page 165 for information. CT0479068 at CT0479105 According to at least the examples above, the QX600 comprises a non-transitory computer readable medium storing instructions thereon, both literally and under the doctrine of equivalents.

BIO-RAD EX.1009.166

Claims	Exemplary Disclosure of Accused Product
[19.c.1.A] obtain cumulative signal data from a digital PCR instrument with a sample volume;	The QX600 comprises a processor that, upon execution of instructions stored on a non-transitory computer readable medium, obtain cumulative signal data from a digital PCR instrument with a sample volume, both literally and under the doctrine of equivalents. For example, operation of the QX600 Droplet Reader involves the operation of a computer which obtains cumulative signal data from a digital PCR instrument (e.g., the QX600 Droplet Reader). *See, e.g.*:

BIO-RAD EX.1009.167

Claims	Exemplary Disclosure of Accused Product
	## Included Items and Additional Components This section identifies the functional items included with your droplet reader purchase, as well as additional components and consumables. For information on items you may need for a full ddPCR cycle, see Appendix E, Ordering Information. Table 5 explains the components that ship with the QX600 Droplet Reader. **Table 5. QX600 Droplet Reader and Included Components** **Component** / **Description** / **Catalog Number** QX600 Droplet Reader — Droplet reading instrument — 12013328 USB cable and power cord — USB cable that connects the computer to the instrument and power cord that connects the instrument to the power source. **Note:** To obtain cables or power cords, contact Bio-Rad technical support. — Included Droplet reader plate holders (2) — Positions and secures the 96-well plate in the droplet reader plate compartment — 12006834 To ensure you have everything you need to complete runs on the QX600 Droplet Reader and analyze your results, Table 6 identifies additional components and accessories that you can purchase separately. **Table 6. Additional Accessories and Consumables** **Item** / **Description** / **Catalog Number** Computer — Computer that connects to the QX600 Droplet Reader for data collection and analysis — 12017458 96-well PCR plates — ddPCR 96-well plates — 12001925 Rainin pipets — 20 µl for sample loading 50 µl for droplet transfer 8-channel, 200 µl for oil — L-20, L8-20 L-50, L8-50 L8-200 CT0479068 at CT0479095

BIO-RAD EX.1009.168

Claims	Exemplary Disclosure of Accused Product
	# Appendix E Ordering Information This appendix contains descriptions and catalog numbers for new or replacement instruments, accessories, and consumables for Bio-Rad's ddPCR products, including your QX600 Droplet Reader. **ddPCR System and Instrument Packages** Table 41 contains information on Bio-Rad Laboratories, Inc. Droplet Digital PCR systems, as well as individual ddPCR instruments and included accessories. **Table 41. ddPCR System and Instrument Packages** | Product	Description	Catalog Number | |---|---|---| | QX200 Droplet Digital PCR System | Includes the QX200 Droplet Generator, QX200 Droplet Reader, computer, software, and consumables | 1864001 | | QX600 Droplet Digital PCR System | Includes the QX200 Droplet Generator, QX600 Droplet Reader, computer, software, and consumables | 17007769 | | QX600 AutoDG™ Droplet Digital PCR System | Includes the Automated Droplet Generator (AutoDG), QX600 Droplet Reader, computer, software, and consumables | 17008371 | | QX200 Droplet Generator | Includes the droplet generator (1), cartridges and gaskets (24 each), cartridge holders (2), and power cord (1) | 1864002 | | QX200 Droplet Reader | Includes the droplet reader (1), plate holders (2), USB cable (1), and power cord (1) | 1864003 | | QX600 Droplet Reader | Includes the droplet reader (1), plate holders (2), USB cable (1), and power cord (1) | 12013328 | | Automated Droplet Generator | Includes the automated droplet generator (1), cooling block accessory (1), oil purge reservoir (1), and power cord (1) | 1864101 | | C1000 Thermal Cycler | Thermal cycler with 384-well reaction module | 1851384 | CT0479068 at CT0479276

BIO-RAD EX.1009.169

Claims	Exemplary Disclosure of Accused Product
	## QX600 Droplet Reader Accessories Table 42 contains information on accessories for the QX600 Droplet Reader. **Table 43. QX600 Droplet Reader Accessories** | Product	Description	Catalog Number
---	---	---
Computer	Computer that connects to the QX600 Droplet Reader for data collection and analysis	12017458
USB cable and power cord	Cable connecting the computer to the instrument and power cord connecting the instrument to the power source **Note:** Contact Bio-Rad Technical Support for replacement information.	Included
Droplet reader plate holders (2)	Used to position the 96-well plate in the droplet reader plate compartment	12006834	 CT0479068 at CT0479278 The QX600 ddPCR workflow comprises combining an analyte-specific reagent mixture with a sample (e.g., DNA or RNA) in a 96-well plate or tubes. *See, e.g.*:

BIO-RAD EX.1009.170

Claims	Exemplary Disclosure of Accused Product
	ddPCR Workflow The ddPCR process adheres to the following workflow: ■ You prepare your samples for PCR by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermix. ■ A droplet generator fractionates a sample into~20,000 nanoliter-sized droplets with target and background DNA distributed randomly into the droplets during the partitioning process. ■ Following droplet generation, the droplets are run through a thermal cycler, which performs PCR amplification of the nucleic acid target in each individual droplet. ■ A droplet reader reads each droplet to determine the fraction of positive droplets in the original sample and uses Poisson statistical formulas to determine the absolute quantity. **Note:** Positive droplets containing at least one copy of the target DNA molecule exhibit increased fluorescence compared to negative droplets. CT0479068 at CT0479089

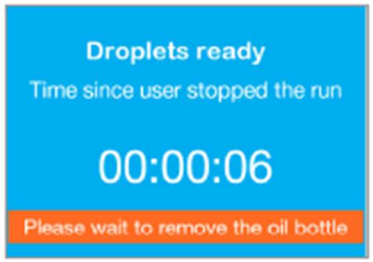
BIO-RAD EX.1009.171

Claims	Exemplary Disclosure of Accused Product
	Preparing the Sample This section contains suggested best practices and procedures for preparing the sample for the droplet generation run. Note the following general recommendations for best practices in preparing the PCR reaction. ■ Combine ddPCR supermix, 20x primers and probe, and DNA sample according to the instructions in the supermix product insert. ■ Use standard lab precautions to avoid contamination of the reaction mix and sample. Wear gloves, work in a clean area (such as a PCR hood) and use clean pipets and low protein binding tubes. ■ Use only the PCR supermixes recommended in Appendix C, Ordering Information. ■ Approved supermixes contain reagents required for droplet generation. Follow instructions in the product inserts to prepare the samples for droplet generation. ■ Using unapproved supermixes can harm the instrument and void the warranty. ■ Ensure the supermix concentration is 1x in final reaction. ■ Prepare the sample in low-bind Eppendorf tubes or ddPCR 96-Well Plate (catalog #12001925). ■ The concentration of intact human genomic DNA should be less than 66 ng per 20 µl reaction. If using higher concentrations, digest DNA with a restriction endonuclease that does not cut target or reference amplicons. CT0479286 at CT0479323 After the droplet generator fractionates the combination into at least one sample volume (e.g., a droplet), the 96-well plate or tubes holding the at least one sample volume (e.g., a droplet) is inserted into the digital PCR instrument (e.g., a QX600 Droplet Reader) for reading. *See e.g.*:

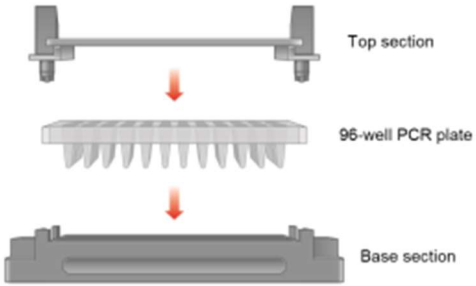
BIO-RAD EX.1009.172

Claims	Exemplary Disclosure of Accused Product
	ddPCR Workflow The ddPCR process adheres to the following workflow: ■ You prepare your samples for PCR by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermix. ■ A droplet generator fractionates a sample into~20,000 nanoliter-sized droplets with target and background DNA distributed randomly into the droplets during the partitioning process. ■ Following droplet generation, the droplets are run through a thermal cycler, which performs PCR amplification of the nucleic acid target in each individual droplet. ■ A droplet reader reads each droplet to determine the fraction of positive droplets in the original sample and uses Poisson statistical formulas to determine the absolute quantity. **Note:** Positive droplets containing at least one copy of the target DNA molecule exhibit increased fluorescence compared to negative droplets. CT0479068 at CT0479089

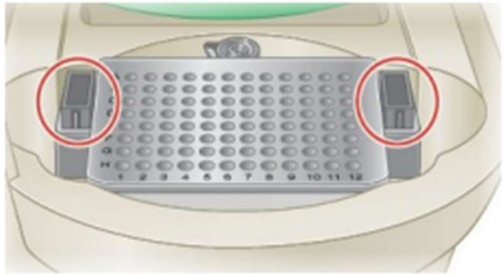
BIO-RAD EX.1009.173

Claims	Exemplary Disclosure of Accused Product
	When the run is finished, the following blue pulsing screen appears and the timer begins counting forward to show the time elapsed since the run concluded.  Messages to the user appear below the timer. **Important:** Do not remove the oil bottle until the unused oils are returned and the highlighted message disappears. 3. When the door on the instrument unlocks, remove the droplet plate from the instrument. 4. Remove and discard used or empty single-use consumables, such as cartridges, pipet tips, and plates. 5. Seal the plate for thermal cycling and droplet reading. For information, refer to the user guides for sealing, thermal cycling, and droplet reading instruments. CT0479286 at CT0479338

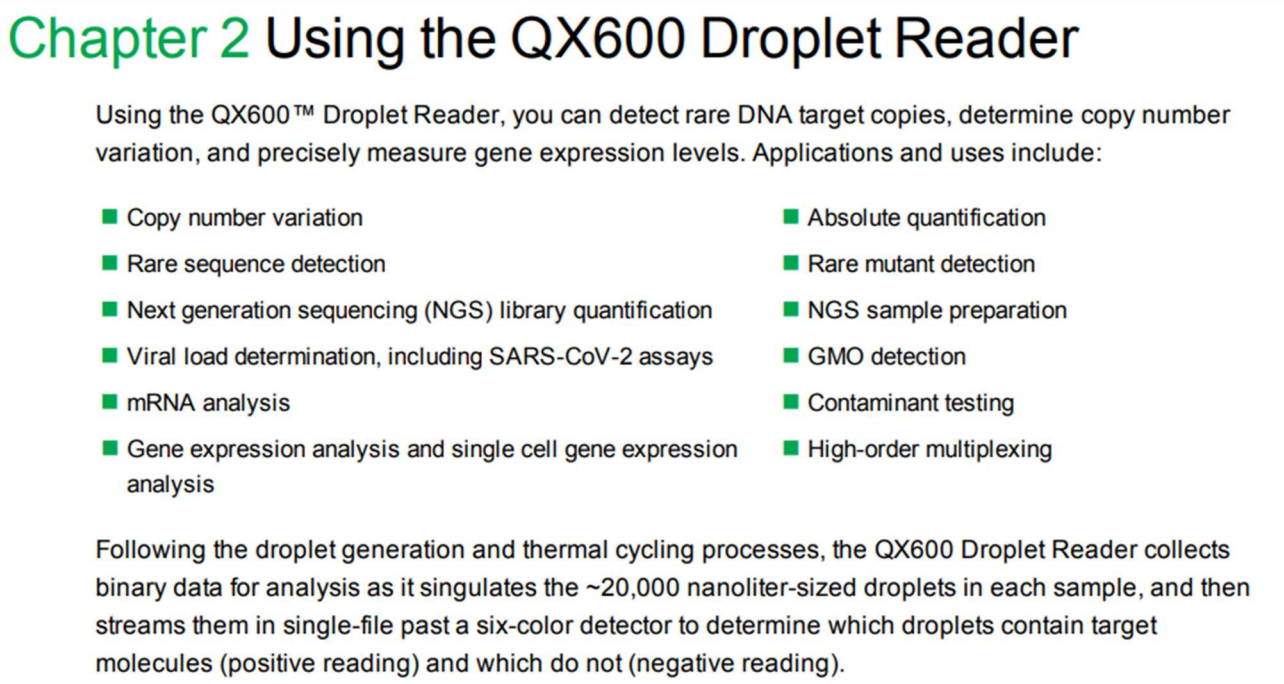
BIO-RAD EX.1009.174

Claims	Exemplary Disclosure of Accused Product
	Assembling a Plate The QX600 Droplet Reader accepts a solid 96-well plate that fits into a secure holder. You must assemble the plate components before you can insert the plate into the QX600 Droplet Reader.Three separate components comprise the plate: ■ A base section to hold the plate ■ The plate itself ■ A top section to secure the plate  CT0479068 at CT0479114

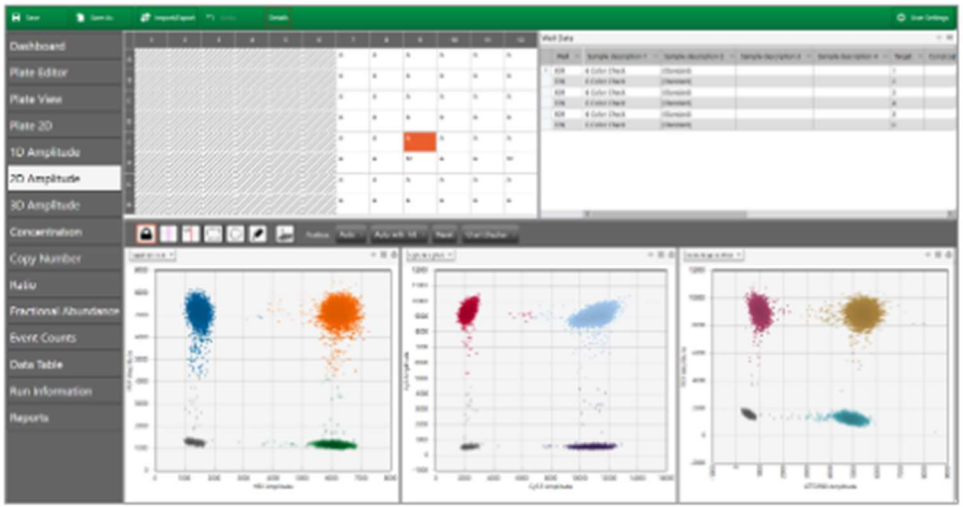
BIO-RAD EX.1009.175

Claims	Exemplary Disclosure of Accused Product
	Important: Make sure the tabs on the plate holder are securely clamped and the plate lays flat. Otherwise, the sampling needle can strike the lid and damage the instrument.  3. Press the button again to close the lid. CT0479068 at CT0479115 The digital PCR instrument (e.g., the QX600 Droplet Reader) receives signals from respective sample volumes (e.g., droplets) through exciting the fluorophores present in the sample volumes (e.g., droplets) to produce electromagnetic signals. The digital PCR instrument (e.g., the QX600 Droplet Reader) and provides this data to the processor (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software). *See, e.g.*:

BIO-RAD EX.1009.176

Claims	Exemplary Disclosure of Accused Product
	 Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). CT0479068 at CT0479090 The signals collect fluorescence information about the multiple fluorophores associated with target analytes within droplets, and thus the signal data is cumulative. *See, e.g.*:

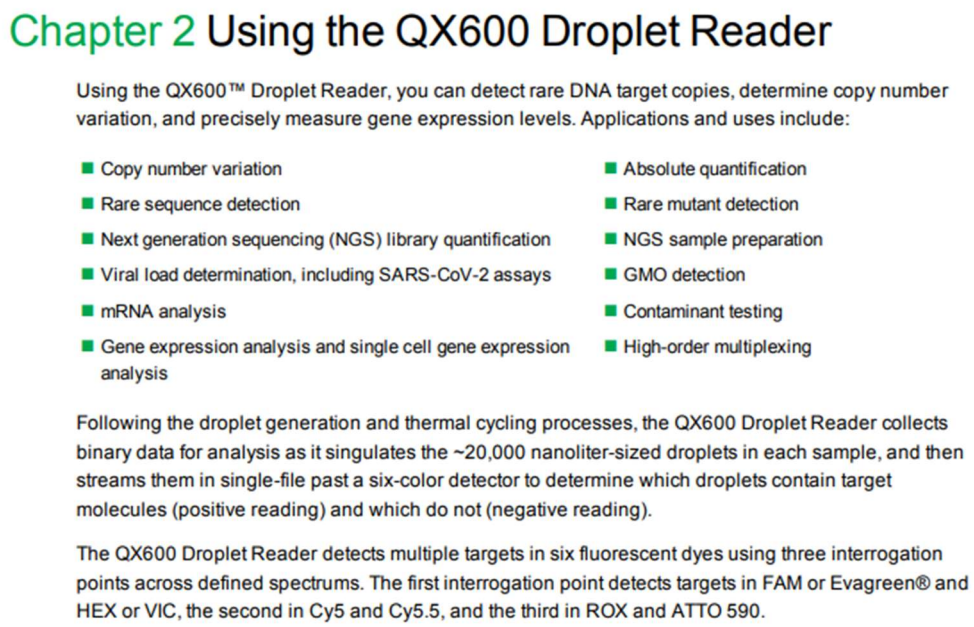
BIO-RAD EX.1009.177

Claims	Exemplary Disclosure of Accused Product
	2D Amplitude For the wells you select in the analysis view of the Plate Editor, the 2D Amplitude window illustrates fluorescence for six targets. The images in this section display data from an experiment involving six targets. Each plot shows individual cluster representations for first and second target positive droplets, droplets containing both targets, and droplets containing neither target. The following graphic shows six colors in standard-size droplets.  CT0479068 at CT0479191 According to at least the examples above, the QX600 comprises a processor (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software) that, upon execution of

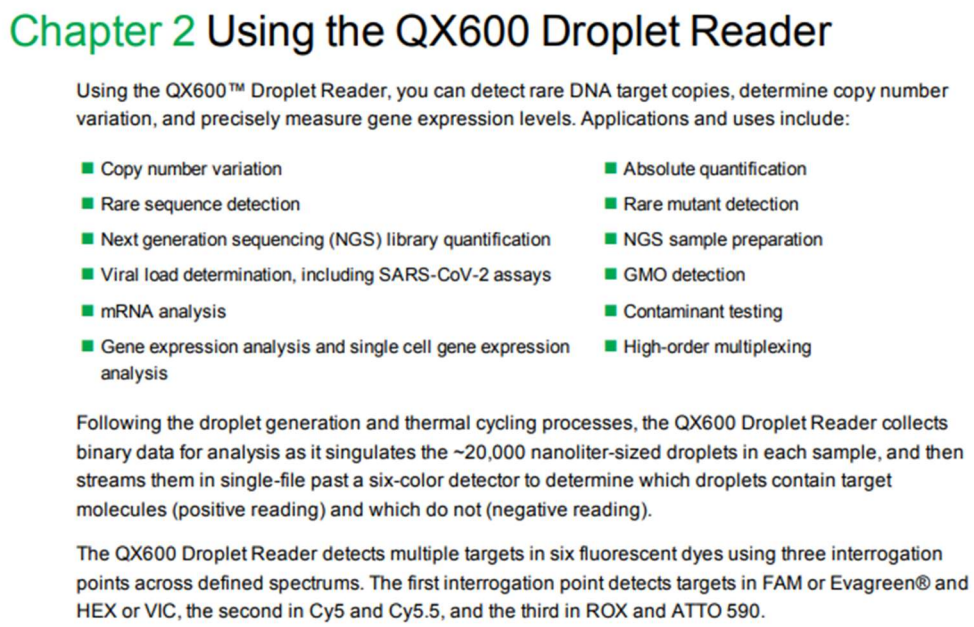
BIO-RAD EX.1009.178

Claims	Exemplary Disclosure of Accused Product
	instructions stored on a non-transitory computer readable medium, obtains cumulative signal data from a digital PCR instrument (e.g., the QX600 Droplet Reader) with a sample volume (e.g., a droplet), both literally and under the doctrine of equivalents.
[19.c.1.B] the digital PCR instrument comprising a light source and a multichannel detector with C channels;	The QX600 comprises a digital PCR instrument, wherein the digital PCR instrument comprises a light source and a multichannel detector with C channels, both literally and under the doctrine of equivalents. For example, the digital PCR instrument (e.g., the QX600 Droplet Reader) comprises a multichannel detector (e.g., the six-color detector) with C (e.g., C=6) channels for detecting electromagnetic signals at the emission wavelengths of six fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590). *See, e.g.*:

BIO-RAD EX.1009.179

Claims	Exemplary Disclosure of Accused Product
	 Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 On information and belief, the multi-channel detector (e.g., the QX600 Droplet Reader's six-color detector) comprises a light source for generating electromagnetic signals to interrogate singulated droplets at the excitation wavelengths of six fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590). *See, e.g.*:

BIO-RAD EX.1009.180

Claims	Exemplary Disclosure of Accused Product
	 Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 According to at least the above examples, the QX600 comprises a digital PCR instrument (e.g., the QX600 Droplet Reader), wherein the digital PCR instrument comprises a light source and a multichannel detector (e.g., the six-color detector) with C (e.g., C=6) channels, both literally and under the doctrine of equivalents.

BIO-RAD EX.1009.181

Claims	Exemplary Disclosure of Accused Product
[19.c.1.C] and the sample volume comprising M fluorescently labeled polynucleotide analytes;	The QX600 comprises a sample volume comprising M fluorescently labeled polynucleotide analytes, both literally and under the doctrine of equivalents. For example, the QX600 ddPCR workflow includes combining a sample (e.g., DNA or RNA) with an analyte-specific reagent mixture (e.g., primers, probes, and Bio-Rad ddPCR supermix). *See, e.g.*: **ddPCR Workflow** The ddPCR process adheres to the following workflow: ■ You prepare your samples for PCR by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermix. ■ A droplet generator fractionates a sample into~20,000 nanoliter-sized droplets with target and background DNA distributed randomly into the droplets during the partitioning process. ■ Following droplet generation, the droplets are run through a thermal cycler, which performs PCR amplification of the nucleic acid target in each individual droplet. ■ A droplet reader reads each droplet to determine the fraction of positive droplets in the original sample and uses Poisson statistical formulas to determine the absolute quantity. **Note:** Positive droplets containing at least one copy of the target DNA molecule exhibit increased fluorescence compared to negative droplets. CT0479068 at CT0479089

BIO-RAD EX.1009.182

Claims	Exemplary Disclosure of Accused Product
	The analyte-specific reagent mixtures (e.g. primers, probes, and Bio-Rad ddPCR supermix) include certain PCR supermixes for probe-based assays, including hydrolysis probe-based assays. *See, e.g.*:

Supermixes

QX Manager Software offers five PCR supermixes. Each supermix is optimized to deliver maximum PCR efficiency and sensitivity for the amplification and detection of DNA and RNA targets.

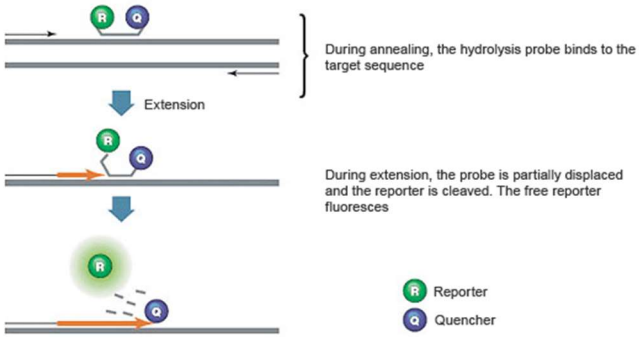
Important: Use of unapproved supermixes can harm the instrument and void the warranty.

ddPCR Supermix	Description
Multiplex Supermix	For use in nucleic acid sample preparations to amplify and detect DNA targets in probe-based experiments involving multiple targets. **Note:** Bio-Rad recommends using ddPCR Multiplex Supermix for Cy5 and Cy5.5 probes assays.
Supermix for Probes (No dUTP)	For use in nucleic acid sample preparations, with sensitivity for the amplification and detection of DNA targets using hydrolysis probe-based assays.
Supermix for Probes	For use in sample preparation with uracil N-glucosylase (UNG) decontamination protocols to prevent the reamplification of carryover PCR products between experiments.
Supermix for Residual DNA Quantification	For use in residual DNA detection.
One-Step RT Advanced Kit for Probe	For use in absolute quantification of target RNA molecules.
EvaGreen® Supermix	For use in gene expression.

BIO-RAD EX.1009.183

Claims	Exemplary Disclosure of Accused Product
	CT0479068 at CT0479138 The analyte-specific reagent mixture includes analyte-specific hybridization probes and multiple fluorophores. Hydrolysis probes fluorescently label polynucleotide analytes (e.g., by cleaving fluorescent reporters during extension). *See, e.g.*:

BIO-RAD EX.1009.184

Claims	Exemplary Disclosure of Accused Product
	Hydrolysis (TaqMan) Probes Hydrolysis assays (TaqMan or 5' nuclease assays) include a sequence-specific, fluorescently labeled oligonucleotide probe in addition to a sequence-specific PCR primer. Hydrolysis assays exploit the 5' → 3' exonuclease activity of certain thermostable polymerases such as *Taq* or *Tth*. The hydrolysis probe is labeled with a fluorescent reporter at the 5' end and a quencher at the 3' end. Commonly used fluorescent reporter-quencher pairs are fluorescein (FAM), which emits green fluorescence, and Black Hole Quencher 1 dye.  **Fig. 2. Hydrolysis probes are hydrolyzed by *Taq* polymerase.** When the hydrolysis probe is intact, the fluorescence of the reporter is quenched due to its proximity to the quencher (Figure 2). The amplification reaction includes a combined annealing/extension step during which the probe hybridizes to the target, and the dsDNA-specific 5' → 3' exonuclease activity of *Taq* or *Tth* cleaves off the reporter. As a result, the reporter is separated from the quencher, resulting in a fluorescence signal that is proportional to the amount of amplified product in the sample. The main advantages of using hydrolysis probes are high specificity, a high signal-to-noise ratio, and the ability to perform multiplex reactions. The disadvantages are that the initial cost of the probe may be high, and the assay design may not be trivial. CT0481963 at CT0481965–CT0481966

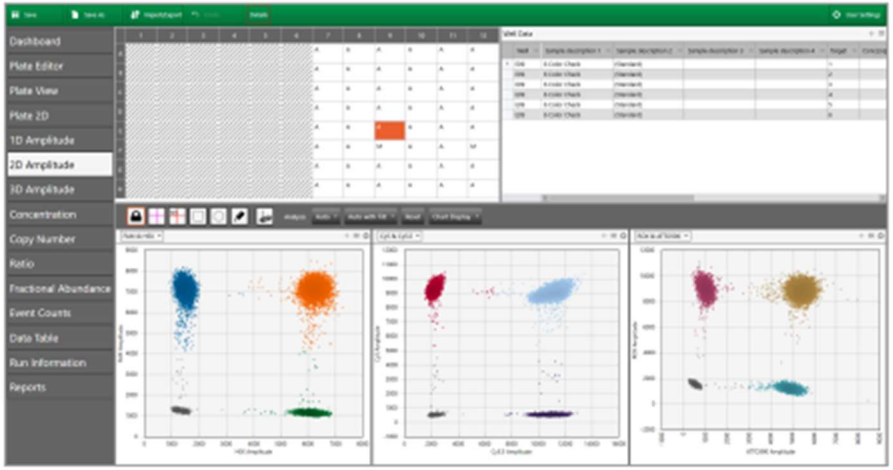
BIO-RAD EX.1009.185

Claims	Exemplary Disclosure of Accused Product
	According to at least the above examples, the QX600 comprises a sample volume (e.g. droplet) comprising M fluorescently labeled polynucleotide analytes, both literally and under the doctrine of equivalents.
[19.c.2] apply a decoding matrix to the cumulative signal data to unambiguously determine the presence or absence of at least each of the M fluorescently labeled polynucleotide analytes	The processor applies a decoding matrix to the cumulative signal to unambiguously detect the presence or absence of at least each of the M fluorescently labeled polynucleotide analytes, both literally and under the doctrine of equivalents. For example, the processor (e.g., of a computer connected to the QX600 Droplet Reader running the QX Manager Software) presents analysis data in different formats, including chromatograms, wherein threshold lines are used to unambiguously distinguish positive or negative droplet counts as indications of the presence or absence of fluorescently labeled analytes. *See, e.g.*:

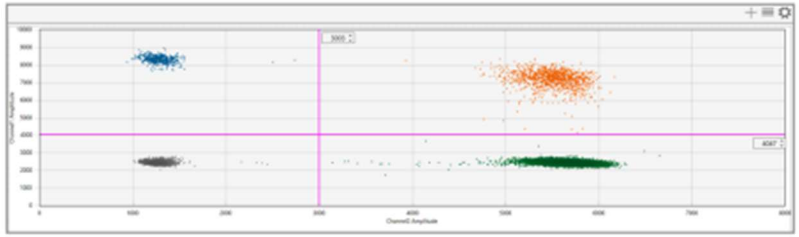
BIO-RAD EX.1009.186

Claims	Exemplary Disclosure of Accused Product
	# Chapter 8 Data Analysis Methodology As summarized in Chapter 7, Data Analysis Module Overview, the software presents analysis data in several different formats. When you open the data file in the Analysis module, you can view the data with the automatic analysis thresholds and calculations or you can choose different options to recalculate, replot, or recolor the results. Data is shown in the following formats: ■ Amplitude, which shows each droplet in each well in a scatter plot, in views ■ Data visualizations, which show estimated target molecule concentration, ratio, and fractional abundance of one target against another target based on selected error model and confidence interval **Note:** Copy number variation is also calculated if the user ran a CNV experiment. ■ Event counts, which are precise counts of positive and negative droplets for each target in each well CT0479068 at CT0479180

BIO-RAD EX.1009.187

Claims	Exemplary Disclosure of Accused Product
	2D Amplitude For the wells you select in the analysis view of the Plate Editor, the 2D Amplitude window illustrates fluorescence for six targets. The images in this section display data from an experiment involving six targets. Each plot shows individual cluster representations for first and second target positive droplets, droplets containing both targets, and droplets containing neither target. The following graphic shows six colors in standard-size droplets.  CT0479068 at CT0479191 The processor (e.g., of a computer connected to the QX600 Droplet Reader running the QX Manager Software) utilizes thresholds. *See, e.g.*:

BIO-RAD EX.1009.188

Claims	Exemplary Disclosure of Accused Product
	Note: Well data must meet certain quality metrics (minimum of 10,000 droplets) before QX Manager Software will automatically calculate a threshold above which droplets are considered positive. You can use the manual thresholding tools to ensure correct quantification of positive and negative droplets. **To use the 2D amplitude tools** 1. Open your data file in the Analysis module and select the 2D Amplitude tab. 2. Choose a threshold line or cluster option. **Note:** Use the cluster option when the data is not orthogonal and the threshold lines do not adequately divide the populations of droplets. ■ To adjust threshold lines, select a threshold line type () and then click in the plot to apply crosshair lines. Use any of the following methods: □ Drag the lines horizontally or vertically in the plot area. □ Enter a new threshold value in the boxes at the end of each threshold line. □ Tap the up and down arrows to incrementally adjust the numbers.  CT0479068 at CT0479192

BIO-RAD EX.1009.189

Claims	Exemplary Disclosure of Accused Product
	The processor (e.g., of a computer connected to the QX600 Droplet Reader running the QX Manager Software) recognizes certain assay types, including Amplitude Multiplex assay types. *See, e.g.*: Appendix A Experiment Examples This section contains setup and analysis information for the following sample experiments: ■ Direct Quantification, using the Amplitude Multiplex assay type ■ Copy Number Variation, using the Probe Mix Triplex assay type ■ Drop-Off, using the Basic Drop-off Assay type for genome edit detection ■ Direct Quantification, using the Advanced Classification Method assay type CT0479068 at CT0479250 When analyzing Amplitude Multiplex assay types, the processor (e.g., of a computer connected to the QX600 Droplet Reader running the QX Manager Software) uses the thresholds to set the boundaries of discrete signal intensity levels discrete channel intensity levels (e.g., None, Hi, Lo). The processor (e.g., of a computer connected to the QX600 Droplet Reader running the QX Manager Software) assigns up to M=12 targets to the discrete channel intensity levels across the QX600's six channels. *See, e.g.*:

BIO-RAD EX.1009.190

Claims	Exemplary Disclosure of Accused Product

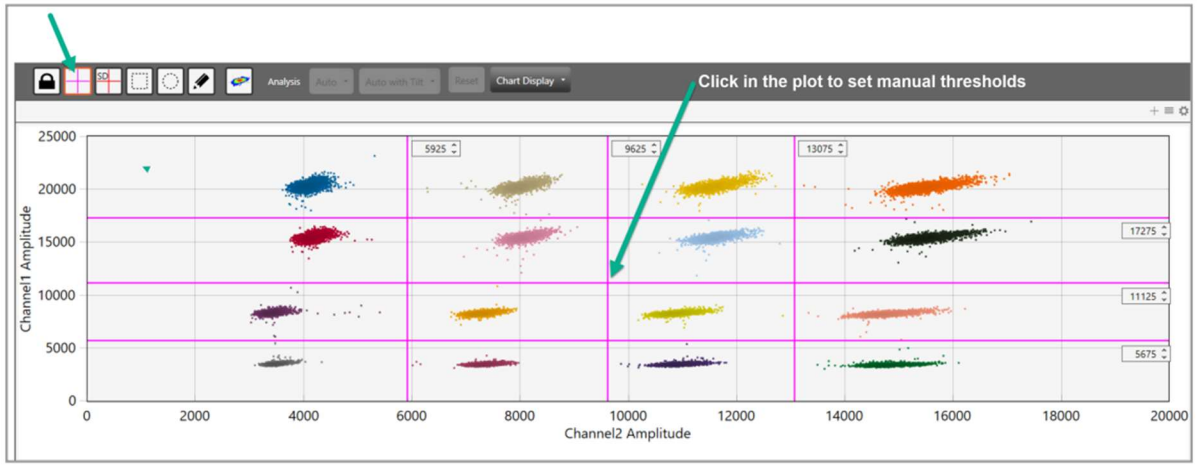
Table 17. Fluorophore options

Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4	Signal Ch5	Signal Ch6
Single target per channel, for1 to 6 targets	FAM EvaGreen® None	HEX VIC None	Cy5 None	Cy5.5 None	ROX None	ATTIO590 None
Amplitude multiplex, for 1 to 12 targets	FAM Hi FAM Lo EvaGreen® Hi EvaGreen® Lo None	HEX Hi HEX Lo VIC Hi VIC Lo None	Cy5 Hi Cy 5 Lo None	Cy5.5 Hi Cy 5.5 Lo None	ROX Hi ROX Lo None	ATTO 590 Hi ATTO 590 Lo None
Probe mix triplex, for 9 targets	FAM None	HEX VIC None	Cy5 None	Cy5.5 None	ROX None	ATTO 590 None
Advanced classification	FAM +1-10 EvaGreen® 1 to 10 None	HEX 1 to 10 VIC 1 to 10 None	Cy5 1 to 10 None	Cy5.5: 1 to 10 None	ROX 1 to 10 None	ATTO 590 1 to 10 None

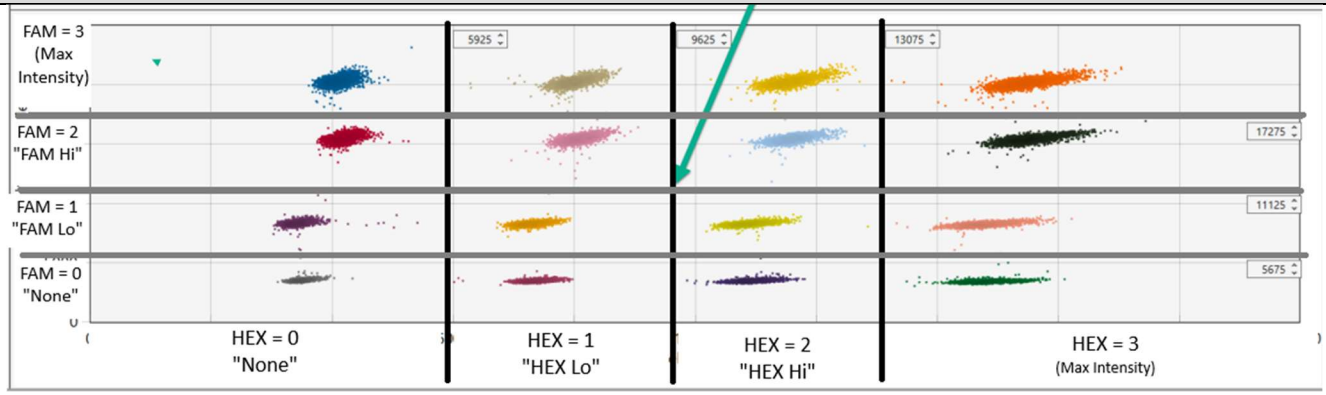
CT0479068 at CT0479141

The boundaries of these discrete channel intensity levels are set by thresholds. *See, e.g.*:

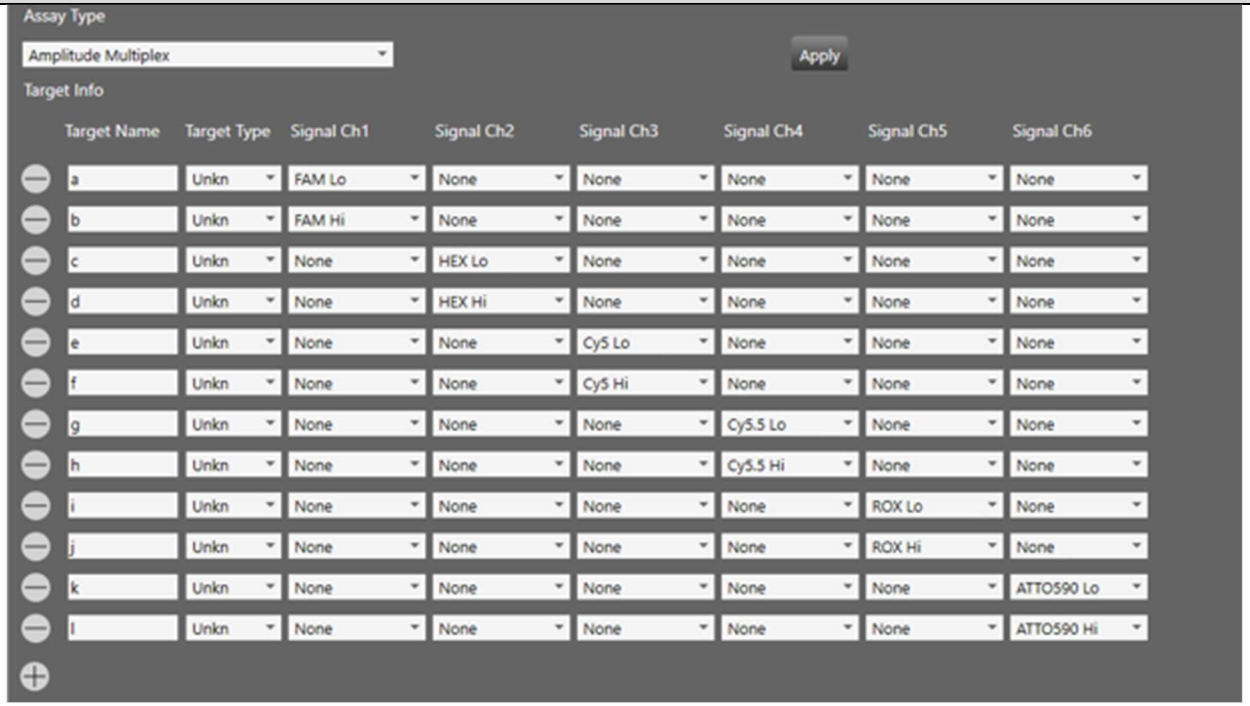
BIO-RAD EX.1009.191

Claims	Exemplary Disclosure of Accused Product
	 CT0479068 at CT0479252 The thresholds above thus are understood to discretize the signal intensity values measured on each of the two channels on each plot into four levels 0, 1, 2, and 3:

BIO-RAD EX.1009.192

Claims	Exemplary Disclosure of Accused Product
	 CT0479068 at CT0479252 (notation of discretized channel signal intensity levels added, threshold line emphasis added) Upon naming of the target analytes in the QX Manager Software, the processor controlled analyzer (e.g., a computer connected to the QX600 Droplet Reader running the QX Manager Software) automatically assigns positive droplet readings within specific regions of the chromatogram to specific combinations of the named target analytes. For example, selecting Amplitude Multiplexing assay type and naming twelve targets a, b, c, d, e, f, g, h, i, j, k, and l:

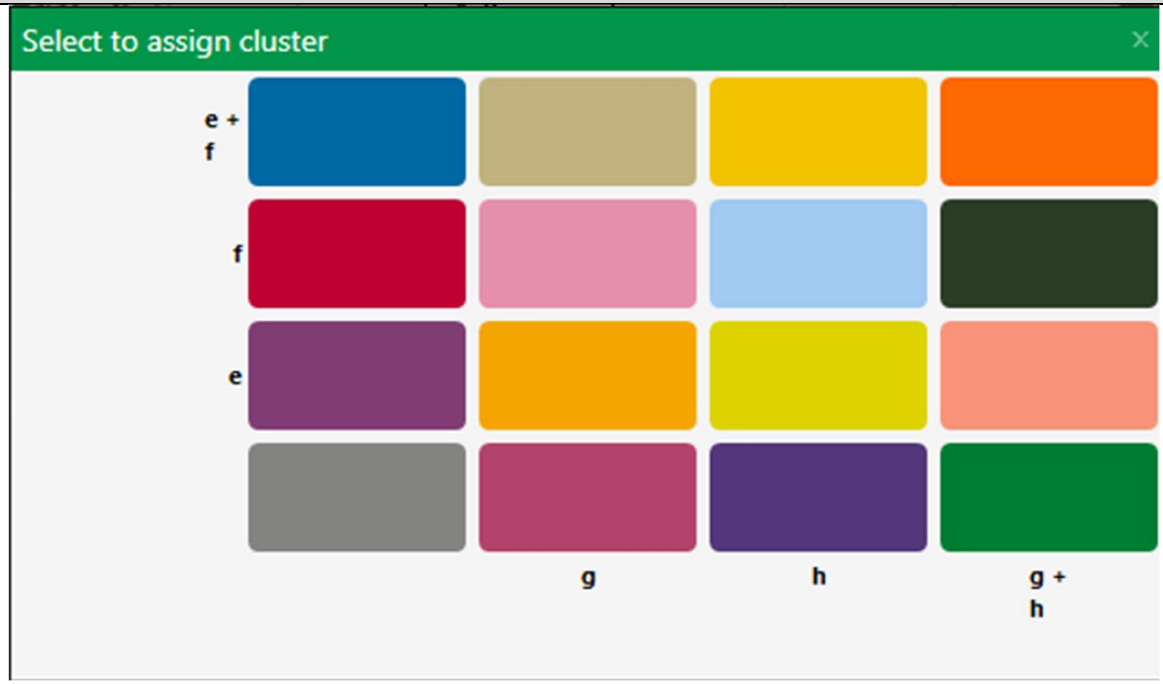
BIO-RAD EX.1009.193

Claims	Exemplary Disclosure of Accused Product
	 CT0478185 When a user attempts to set thresholds on the 2D chromatograms, the QX Manager Software interface reveals the default assignment matrix for amplitude multiplexing assay types. *See e.g.*: For channels 1 and 2 (FAM and HEX):

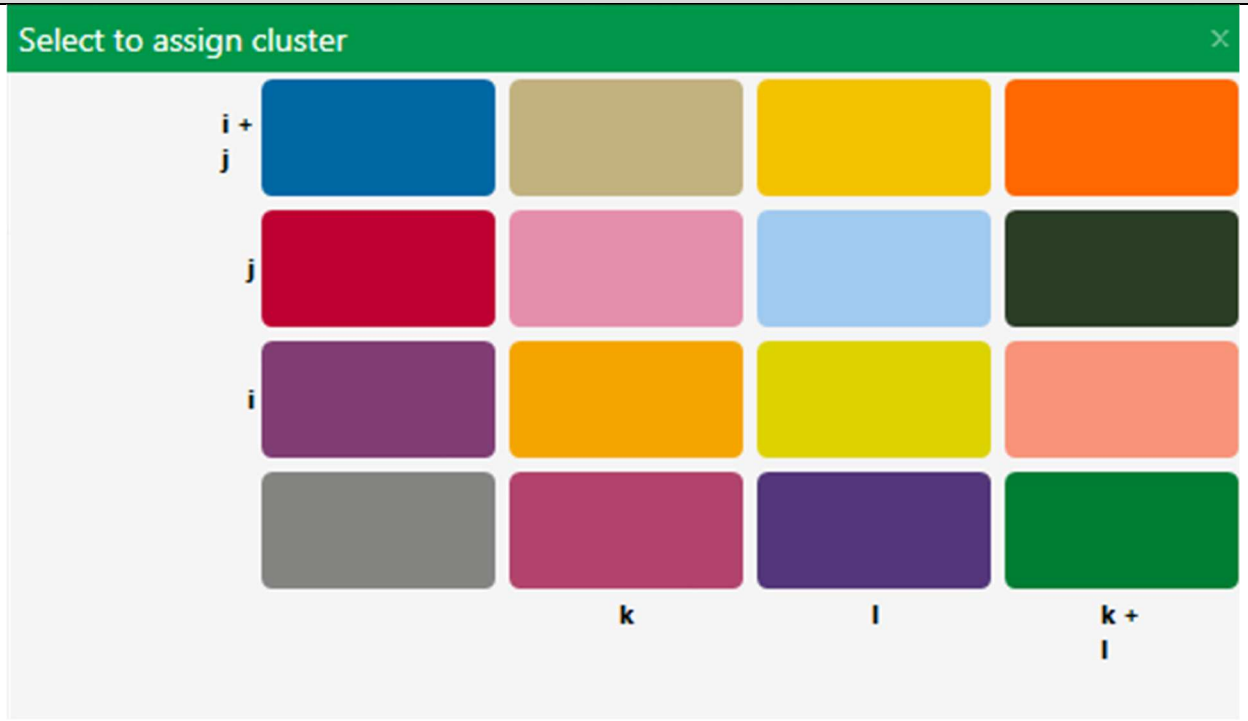
BIO-RAD EX.1009.194

Claims	Exemplary Disclosure of Accused Product
	 CT0478184 For channels 3 and 4 (Cy5 and Cy5.5):

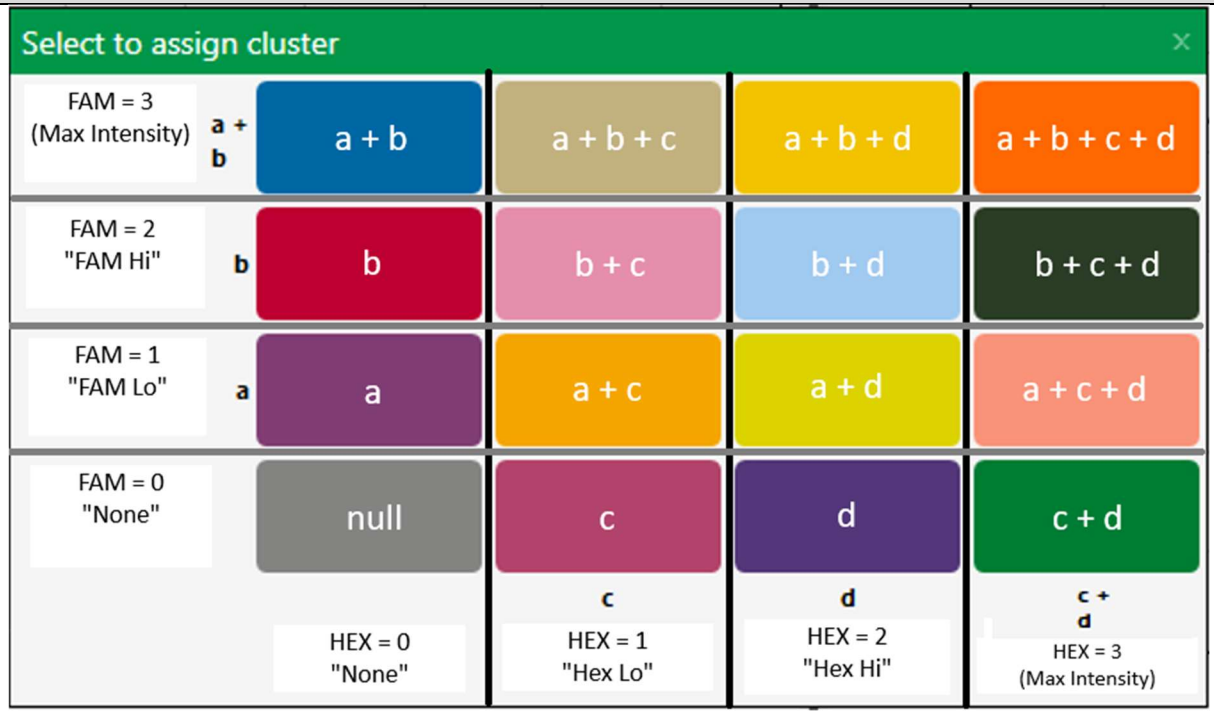
BIO-RAD EX.1009.195

Claims	Exemplary Disclosure of Accused Product
	 CT0478186 For channels 5 and 6 (ROX and ATTO 590):

BIO-RAD EX.1009.196

Claims	Exemplary Disclosure of Accused Product
	 CT0478187 Overlaying the discretized signal intensity levels described above in relation to the threshold lines shows how the specific regions relate to the detected signal intensity levels:

BIO-RAD EX.1009.197

Claims	Exemplary Disclosure of Accused Product
	 CT0478184 (notation of discretized channel signal intensity levels added, threshold line emphasis added, white text indicating each assigned specific combination of analytes added) On information and belief, the processor (e.g., of a computer connected to the QX600 Droplet Reader running the QX Manager Software), applies a decoding matrix to assign the above target analyte combinations to the available regions formed by the discretized signal channel levels, and then droplet data with signal channel levels falling within a given discretized signal channel region is associated with the target analyte combination assigned to that region. Alternatively, or in addition, the processor (e.g., of a computer connected to the QX600 Droplet Reader

BIO-RAD EX.1009.198

<table>
<tr><th>Claims</th><th>Exemplary Disclosure of Accused Product</th></tr>
<tr><td></td><td>running the QX Manager Software) uses a function substantially the same as applying a decoding matrix to the cumulative signal (e.g., assigning the droplet data to target analyte combinations according to how fluorophore intensity levels in the droplet data relate to defined thresholds), in the same way of using cumulative signal data based on the first, second, third, and fourth electromagnetic signals to achieve the same result of unambiguously detecting the presence or absence of at least each of M analytes.

The decoding matrix for channels 1 and 2 may be represented as the following:

<table>
<tr><th>Analyte</th><th>FAM</th><th>HEX</th></tr>
<tr><td>a</td><td>1</td><td>0</td></tr>
<tr><td>b</td><td>2</td><td>0</td></tr>
<tr><td>c</td><td>0</td><td>1</td></tr>
<tr><td>d</td><td>0</td><td>2</td></tr>
</table>

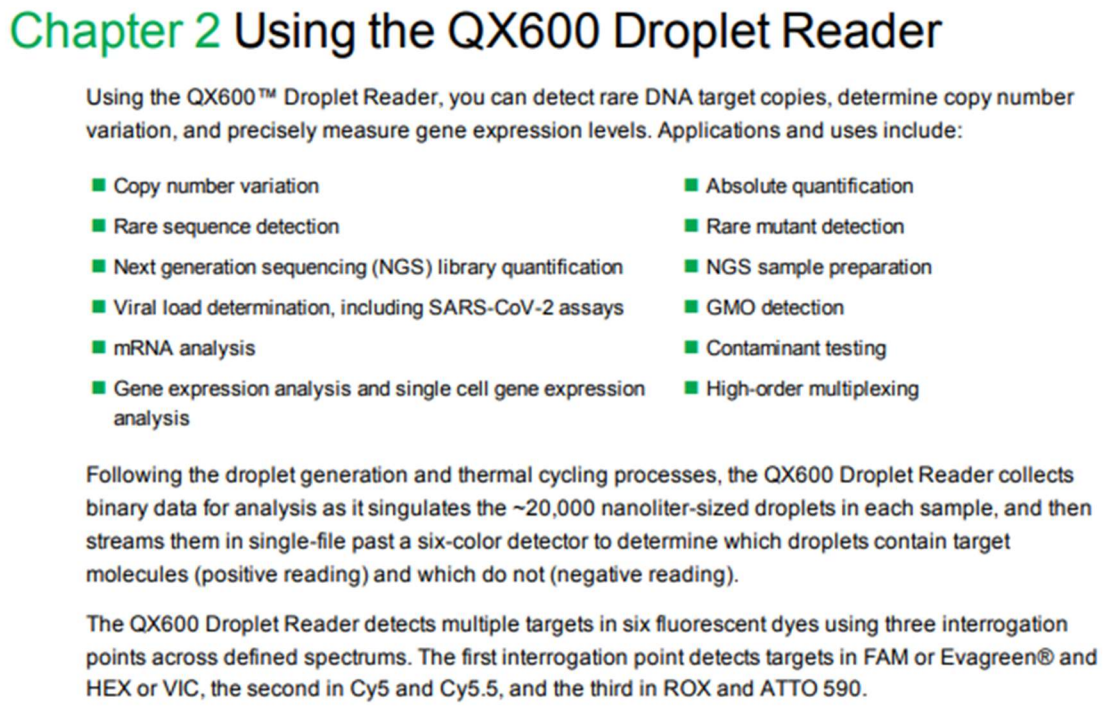
Expanding this same analysis to the default target combination assignments to all six channels as provided by the QX Manager interface, the following larger decoding table covering all twelve target analytes may be derived:

<table>
<tr><th>Analyte</th><th>FAM</th><th>HEX</th><th>Cy5</th><th>Cy5.5</th><th>ROX</th><th>ATTO 590</th></tr>
<tr><td>a</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>b</td><td>2</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>c</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>d</td><td>0</td><td>2</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>e</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>f</td><td>0</td><td>0</td><td>2</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>g</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td></tr>
</table>
</td></tr>
</table>

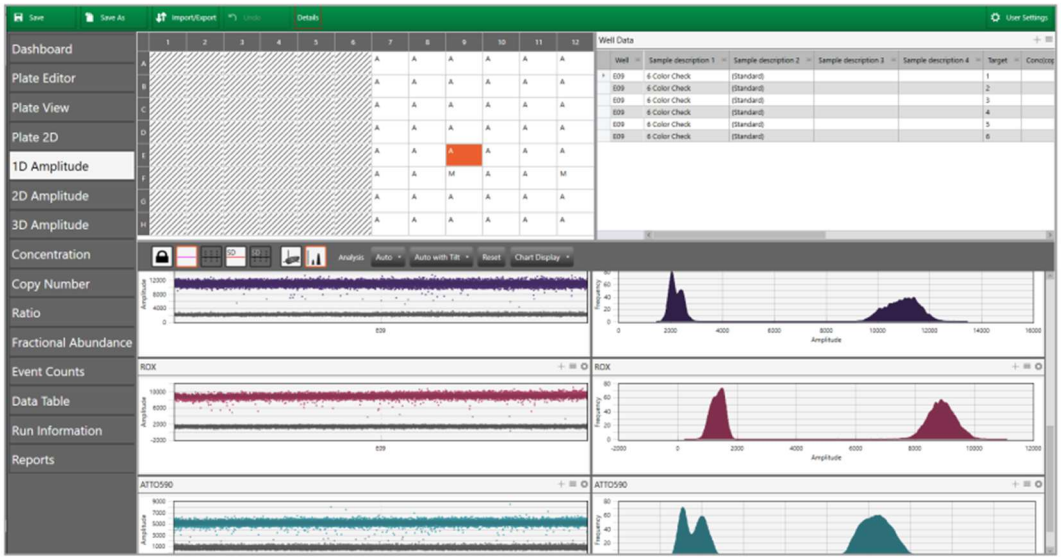
BIO-RAD EX.1009.199

Claims	Exemplary Disclosure of Accused Product
	<table><tr><td>**h**</td><td>0</td><td>0</td><td>0</td><td>2</td><td>0</td><td>0</td></tr><tr><td>**i**</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td></tr><tr><td>**j**</td><td>0</td><td>0</td><td>0</td><td>0</td><td>2</td><td>0</td></tr><tr><td>**k**</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td></tr><tr><td>**l**</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>2</td></tr></table> According to at least the examples above, the processor (e.g., of a computer connected to the QX600 Droplet Reader running the QX Manager Software) applies a decoding matrix to the cumulative signal (e.g. associates positive droplet detections to target analytes according to a decoding matrix) to unambiguously detect the presence or absence of at least each of M fluorescently labeled polynucleotide analytes, both literally and under the doctrine of equivalents.
[19.c.2.A] by associating, for each fluorescently labeled polynucleotide analyte, a first value in a first component of the cumulative signal data and a second value in a second component of the cumulative signal data wherein each first value is an intensity or range of intensities and each	The processor applies the decoding matrix to the cumulative signal data by associating, for each fluorescently labeled polynucleotide analyte, a first value in a first component of the cumulative signal data and a second value in a second component of the cumulative signal data wherein each first value is an intensity or range of intensities and each second value is a wavelength or a range of wavelengths, both literally and under the doctrine of equivalents. For example, as explained in [19.c.2] above, the processor (e.g., of a computer connected to the QX600 Droplet Reader running the QX Manager Software) associates signal intensity levels with certain combinations of analytes. The QX600 Droplet Reader collects intensity measurements from three interrogation points covering six fluorophore wavelength channels (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590). *See. e.g.*:

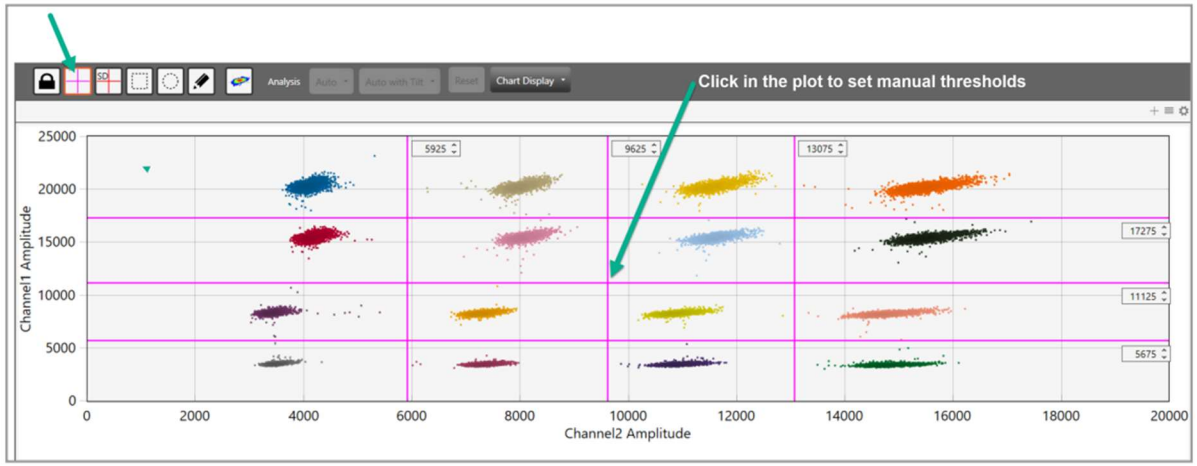
BIO-RAD EX.1009.200

Claims	Exemplary Disclosure of Accused Product
second value is a wavelength or a range of wavelengths,	 CT0479068 at CT0479090 The QX600 Droplet Reader cumulative signal includes signal intensity (e.g., amplitude) values for each fluorophore wavelength. *See e.g.*:

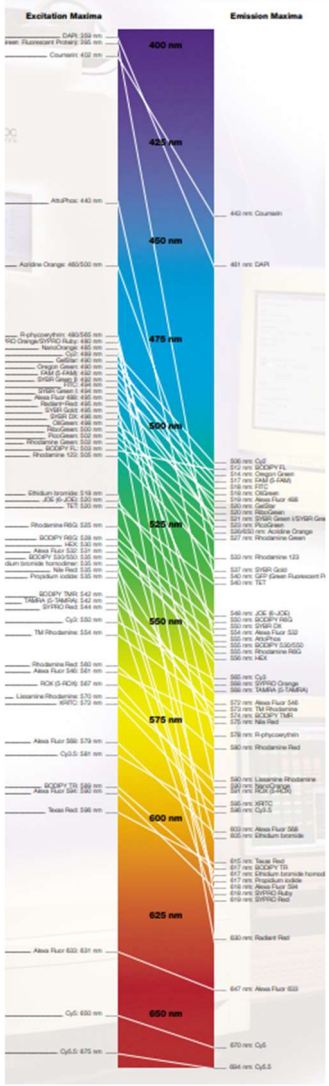
BIO-RAD EX.1009.201

Claims	Exemplary Disclosure of Accused Product
	1D Amplitude Use the 1D Amplitude analysis window to view droplet amplitudes for each channel, in selection order, for each well selected in the Well Selector. The following graphic shows six colors in standard-size droplets.  CT0479068 at CT0479186

BIO-RAD EX.1009.202

Claims	Exemplary Disclosure of Accused Product
	 CT0479068 at CT0479252 Each of the fluorophores has a specific emission wavelength or a range of wavelengths. The emission wavelength for FAM is 517 nm, the emission wavelength for HEX is 556 nm, the emission wavelength for Cy5 is 670 nm, the emission wavelength for Cy5.5 is 694 nm, the emission wavelength for ROX is 591 nm, and the emission wavelength for ATTO 590 is 622 nm. *See, e.g.*:

BIO-RAD EX.1009.203

Claims	Exemplary Disclosure of Accused Product
	

BIO-RAD EX.1009.204

Claims	Exemplary Disclosure of Accused Product
	CT0479364 at CT0479364

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ATTO-TEC

Revised: 2023-11-06

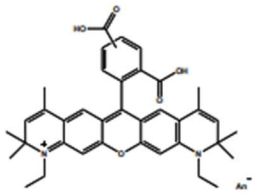
Product Information: ATTO 590

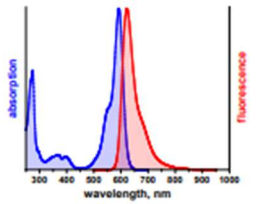


ATTO 590 is a novel fluorescent label belonging to the class of Rhodamine dyes. The dye is designed for application in the area of life science, e.g. labeling of DNA, RNA or proteins. Characteristic features of the label are strong absorption, high fluorescence quantum yield, high thermal and photo-stability, and very little triplet formation. The dye is highly suitable for single-molecule detection applications and high-resolution microscopy.
As supplied **ATTO 590** consists of a mixture of two isomers with practically identical absorption and fluorescence. On request the dye can also be supplied as single isomer.

Optical data of the carboxy derivative *(in PBS, pH 7.4):*

λ_{abs}	=	593 nm
ε_{max}	=	1.2 x 10^5 M^{-1} cm^{-1}
λ_{fl}	=	622 nm
η_{fl}	=	80 %
τ_{fl}	=	3.7 ns
CF_{260}	=	0.39
CF_{280}	=	0.43





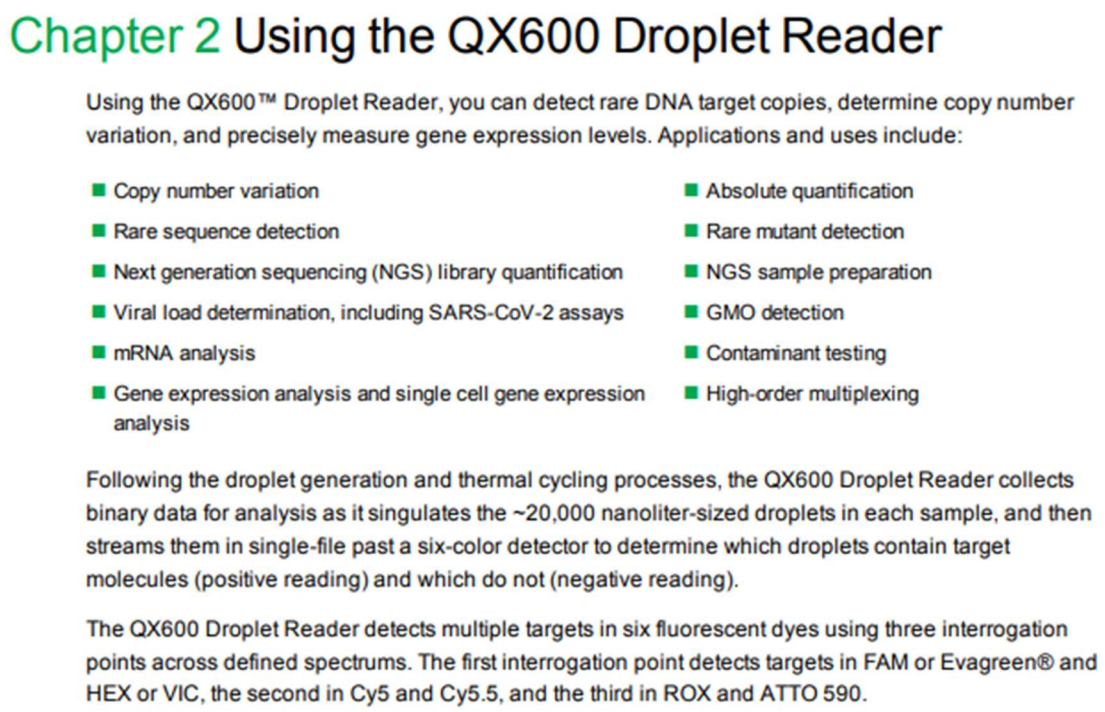
Spectra available in digitized form (excel file) on
http://www.atto-tec.com

CT0479362 at CT0479362

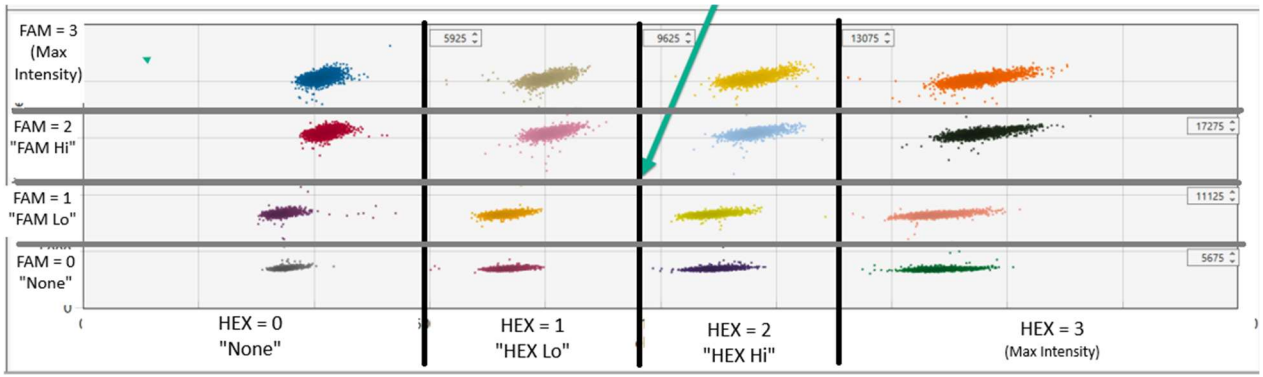
BIO-RAD EX.1009.205

Claims	Exemplary Disclosure of Accused Product
	According to at least the above examples, the processor (e.g., a computer connected to the QX600 Droplet Reader for data collection and analysis) applies the decoding matrix (e.g. assigns target analyte combinations to discrete signal channel levels and associates droplet data with target analyte combinations according to signal channel levels within the droplet data) to the cumulative signal data by associating for each analyte, a first value (e.g., a FAM fluorescence intensity value), in a first component of the cumulative signal and a second value (e.g., a FAM emission wavelength value), in a second component of the cumulative signal, wherein each first value (e.g. a FAM fluorescence intensity value, a HEX fluorescence intensity value, a Cy5 fluorescence intensity value, a Cy5.5 fluorescence intensity value, a ROX fluorescence intensity value, an ATTO 590 fluorescence intensity value), is an intensity or range of intensities and each second value (e.g., a FAM emission wavelength value, a HEX emission wavelength value, a Cy5 emission wavelength value, a Cy5.5 emission wavelength value, a ROX emission wavelength value, an ATTO 590 emission wavelength value) is a wavelength or a range of wavelengths, both literally and under the doctrine of equivalents.
[19.c.2.B] wherein for a positive integer M, M=C*log2(F+1),	The processor makes determinations wherein, for a positive integer M, M=C*log2(F+1), both literally and under the doctrine of equivalents. For example, where intensity data is collected from all six channels the number of second values, C (e.g., fluorophore wavelengths) is six. *See. e.g.*:

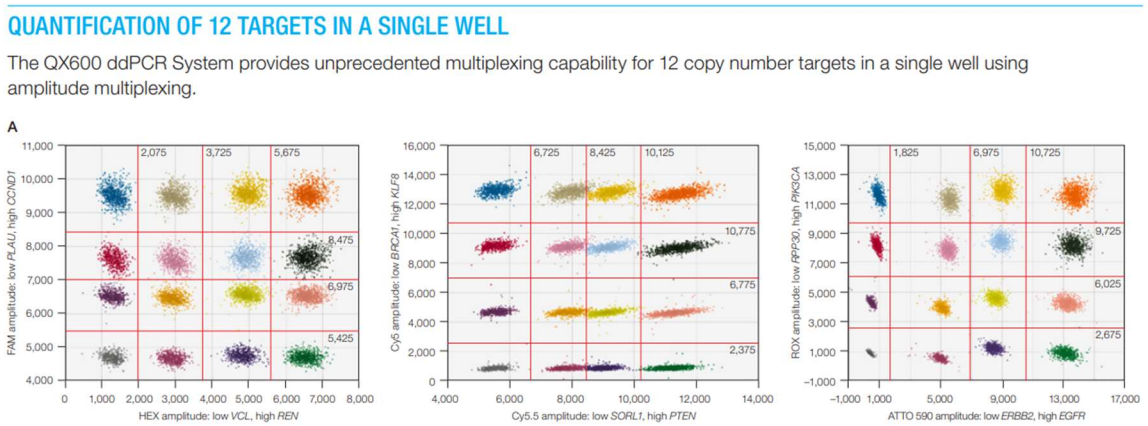
BIO-RAD EX.1009.206

Claims	Exemplary Disclosure of Accused Product
	 CT0479068 at CT0479090 The processor (e.g., of a computer connected to the QX600 Droplet Reader running the QX Manager Software) applies a decoding matrix to the cumulative signal (e.g. assigns target analyte combinations to discrete signal channel levels and associates droplet data with target analyte combinations according to signal channel levels within the droplet data), such that maximum cumulative intensity for any channel, F, is three. *See, e.g.*:

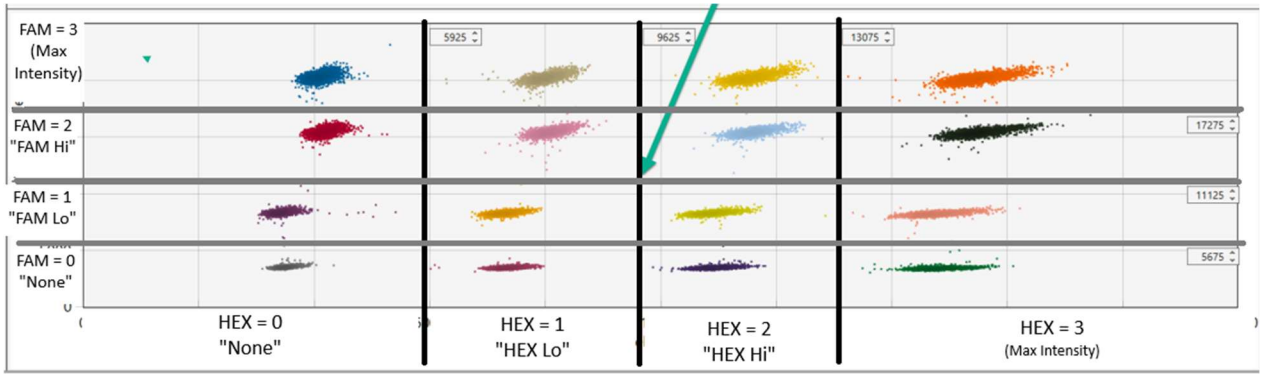
BIO-RAD EX.1009.207

Claims	Exemplary Disclosure of Accused Product
	 CT0479068 at CT0479252 (notation of discretized channel signal intensity levels added, threshold line emphasis added) Plugging C=6 and F=3 into the equation M=C*log2(F+1), calculates an analyte count of twelve: M=C*log2(F+1) M=6*log2(3+1) M=6*log2(4) M=6*2 M=12 This is consistent with the number of analytes unambiguously determined to be present or absent when the QX600 uses six channels, which is twelve analytes. *See e.g.* (twelve analytes unambiguously detected are PLAU, CCND1, VCL, REN, BRCA1, KLF8, SORL1, PTEN, RPP30, PIK3CA, ERBB2,

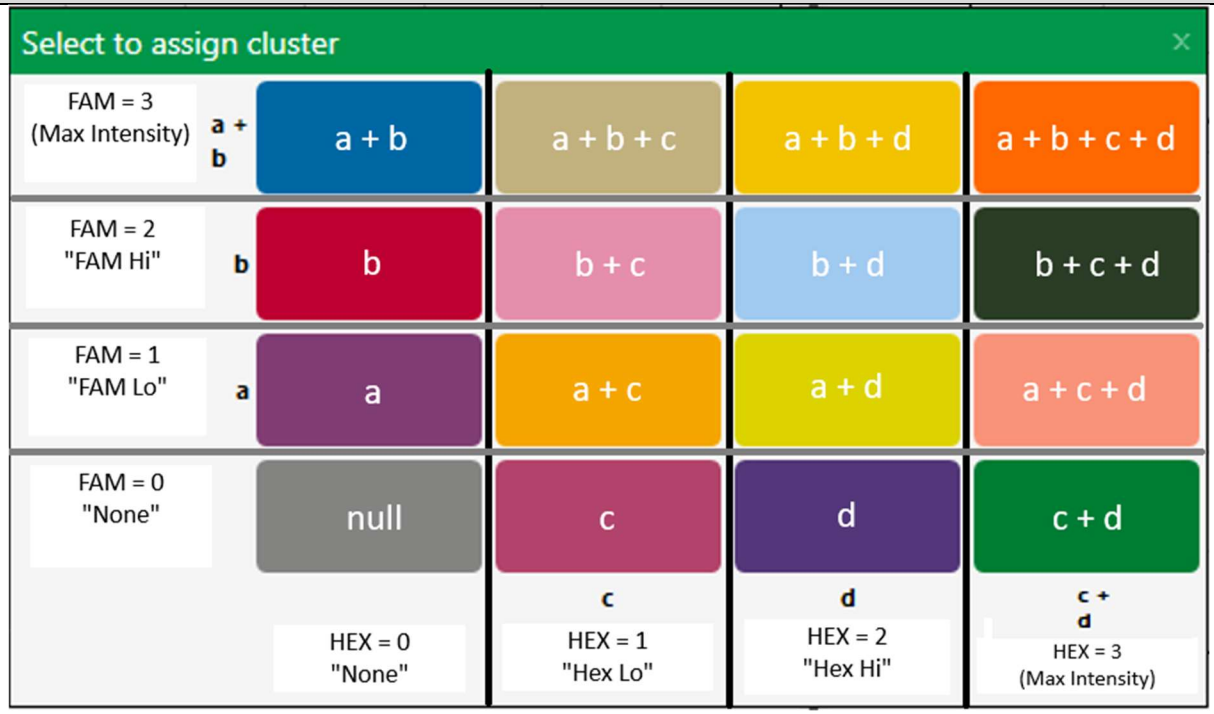
BIO-RAD EX.1009.208

Claims	Exemplary Disclosure of Accused Product
	and EGFR):  CT0479366 at CT0479368 According at least to the above examples, the processor (e.g., of a computer connected to the QX600 Droplet Reader running the QX Manager Software) makes determinations wherein, for a positive integer M (e.g., 12), M=C*log2(F+1) (e.g., 12=6*log2(3+1)), both literally and under the doctrine of equivalents.

BIO-RAD EX.1009.209

Claims	Exemplary Disclosure of Accused Product
[19.c.2.C] F is the maximum cumulative intensity of the first component of the cumulative signal data, for any second value, when all of the analytes are present,	The processor makes determinations wherein F is the maximum cumulative intensity of the first component of the cumulative signal data, for any second value, when all of the analytes are present, both literally and under the doctrine of equivalents. For example, the processor (e.g., of a computer connected to the QX600 Droplet Reader running the QX Manager Software) applies a decoding matrix to the cumulative signal (e.g. assigns target analyte combinations to discrete signal channel levels and associates droplet data with target analyte combinations according to signal channel levels within the droplet data), such that maximum cumulative intensity for any channel, F, is three. *See, e.g.*:  CT0479068 at CT0479252 (notation of discretized channel signal intensity levels added, threshold line emphasis added) Where signal intensity on both the FAM and HEX channels exceeds the threshold for a discrete valuation of three, the detection is classified in the upper right orange region, indicating the presence of all analytes (a + b + c + d). *See, e.g.*:

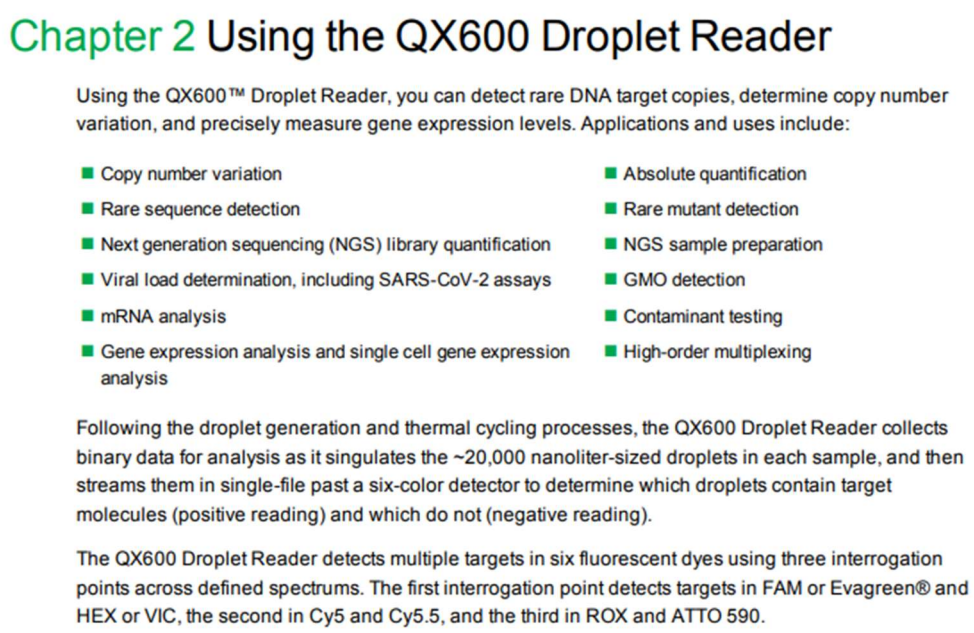
BIO-RAD EX.1009.210

Claims	Exemplary Disclosure of Accused Product
	 CT0478184 (notation of discretized channel signal intensity levels added, threshold line emphasis added, white text indicating each assigned specific combination of analytes added) The orange cluster represents droplets containing all of the analytes of the chromatogram. If signal intensity on all channels (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590) exceeds the threshold for a discrete valuation of three, then the sample would be classified as containing all analytes. According at least to the above examples, the processor (e.g., a computer connected to the QX600

BIO-RAD EX.1009.211

Claims	Exemplary Disclosure of Accused Product
	Droplet Reader running the QX Manager Software) makes determinations wherein F (e.g., F=3) is the maximum cumulative intensity of the first component of the cumulative signal data, for any second value (e.g., intensity at FAM, HEX, Cy5, Cy5.5, ROX, or ATTO 590 emission wavelengths), when all of the analytes are present, both literally and under the doctrine of equivalents.
[19.c.2.D] M, C, and F are each positive integers, and	The processor makes determinations wherein M, C, and F are each positive integers, both literally and under the doctrine of equivalents. For example, the QX600 Droplet Reader collects intensity data from up to six channels (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590) to detect up to 12 targets in amplitude multiplexing mode (i.e., the number of unambiguously detected analytes is twelve, M=12). *See, e.g.*:

BIO-RAD EX.1009.212

Claims	Exemplary Disclosure of Accused Product
	 Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090

BIO-RAD EX.1009.213

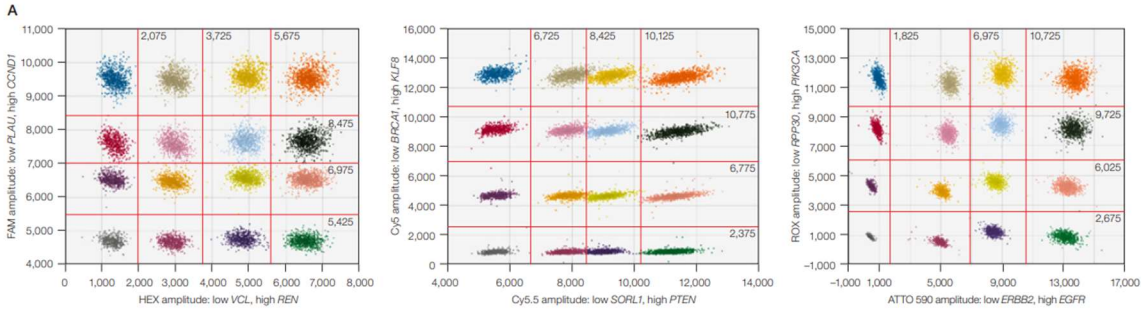
Claims	Exemplary Disclosure of Accused Product
	Table 17. Fluorophore options

Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4	Signal Ch5	Signal Ch6
Single target per channel, for1 to 6 targets	FAM EvaGreen® None	HEX VIC None	Cy5 None	Cy5.5 None	ROX None	ATTIO590 None
Amplitude multiplex, for 1 to 12 targets	FAM Hi FAM Lo EvaGreen® Hi EvaGreen® Lo None	HEX Hi HEX Lo VIC Hi VIC Lo None	Cy5 Hi Cy 5 Lo None	Cy5.5 Hi Cy 5.5 Lo None	ROX Hi ROX Lo None	ATTO 590 Hi ATTO 590 Lo None
Probe mix triplex, for 9 targets	FAM None	HEX VIC None	Cy5 None	Cy5.5 None	ROX None	ATTO 590 None
Advanced classification	FAM +1-10 EvaGreen® 1 to 10 None	HEX 1 to 10 VIC 1 to 10 None	Cy5 1 to 10 None	Cy5.5: 1 to 10 None	ROX 1 to 10 None	ATTO 590 1 to 10 None

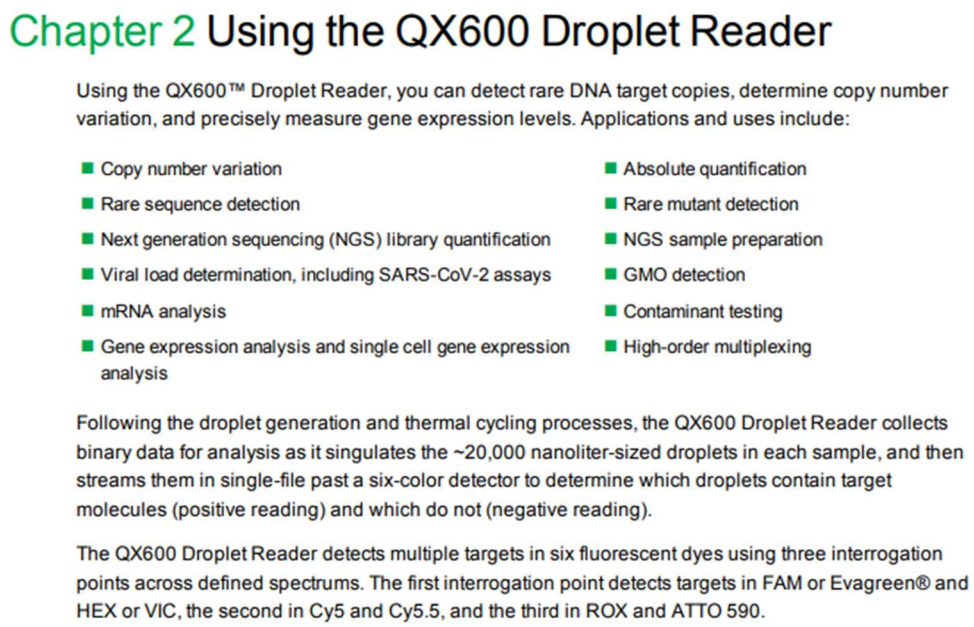
CT0479068 at CT0479141

See also:

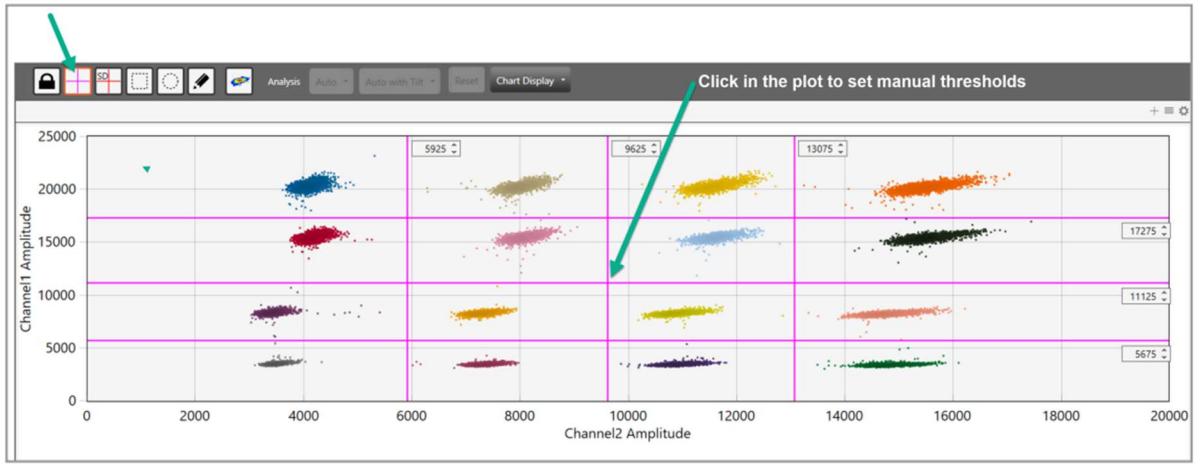
BIO-RAD EX.1009.214

Claims	Exemplary Disclosure of Accused Product
	When the QX600 Droplet Reader collects intensity data from six channels, twelve analytes are unambiguously determined to be present or absent. *See e.g.* (twelve analytes unambiguously detected are PLAU, CCND1, VCL, REN, BRCA1, KLF8, SORL1, PTEN, RPP30, PIK3CA, ERBB2, and EGFR): **QUANTIFICATION OF 12 TARGETS IN A SINGLE WELL** The QX600 ddPCR System provides unprecedented multiplexing capability for 12 copy number targets in a single well using amplitude multiplexing.  CT0479366 at CT0479368 The QX600 Droplet Reader collects intensity data from up to six channels (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590), so the number of second values, C (e.g., fluorophore wavelengths) may be 1, 2, 3, 4, 5, or 6. *See, e.g.*:

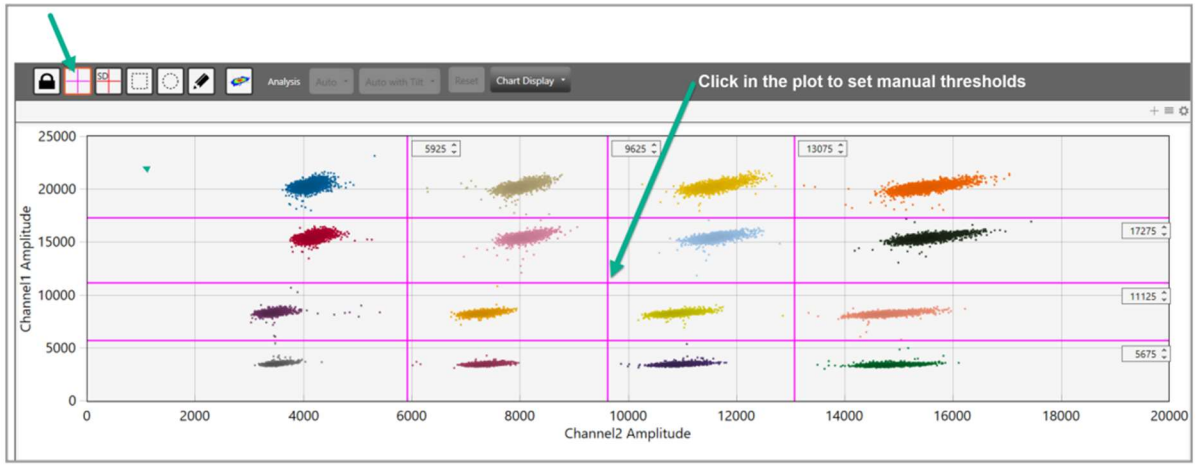
BIO-RAD EX.1009.215

Claims	Exemplary Disclosure of Accused Product
	 CT0479068 at CT0479090 The processor (e.g., of a computer connected to the QX600 Droplet Reader running the QX Manager Software) applies a decoding matrix to the cumulative signal (e.g. assigns target analyte combinations to discrete signal channel levels and associates droplet data with target analyte combinations according to signal channel levels within the droplet data), such that maximum cumulative intensity for any channel, F, is three. *See, e.g.*:

BIO-RAD EX.1009.216

Claims	Exemplary Disclosure of Accused Product
	 CT0479068 at CT0479252 M=12, C=6, and F=3 are each positive integers. According to at least the above examples, the processor makes determinations wherein M, C, and F are each positive integers, both literally and under the doctrine of equivalents.
[19.c.2.E] wherein F+1 is a positive integer and wherein F+1 is a power of 2; and	The processor makes determinations wherein F+1 is a positive integer and wherein F+1 is a power of 2, both literally and under the doctrine of equivalents. The processor (e.g., of a computer connected to the QX600 Droplet Reader running the QX Manager

BIO-RAD EX.1009.217

Claims	Exemplary Disclosure of Accused Product
	Software) applies a decoding matrix to the cumulative signal (e.g. assigns target analyte combinations to discrete signal channel levels and associates droplet data with target analyte combinations according to signal channel levels within the droplet data), such that maximum cumulative intensity for any channel, F, is three. *See, e.g.*:  CT0479068 at CT0479252 F=3 in the above examples, and F+1=4. Four is a positive integer and a power of two, e.g. 2^2=4. According at least to the above examples, the processor (e.g., of a computer connected to the QX600 Droplet Reader running the QX Manager Software) makes determinations wherein F+1 is a positive integer (e.g., 4) and wherein F+1 is a power of 2 (e.g., 4=2^2), both literally and under the doctrine of equivalents.

BIO-RAD EX.1009.218

Claims	Exemplary Disclosure of Accused Product
[19.c.3] plot, on the display, a representation of the first value and the second value;	The QX 600 comprises a display coupled to the processor, wherein the processor plots, on the display, a representation of the first value and the second value, both literally and under the doctrine of equivalents. For example, operation of the QX600 Droplet Reader within the QX600 System involves the operation of additional components (e.g., a computer connected to the QX600 Droplet Reader for data collection and analysis). *See, e.g.*:

BIO-RAD EX.1009.219

Claims	Exemplary Disclosure of Accused Product
	## Included Items and Additional Components This section identifies the functional items included with your droplet reader purchase, as well as additional components and consumables. For information on items you may need for a full ddPCR cycle, see Appendix E, Ordering Information. Table 5 explains the components that ship with the QX600 Droplet Reader. **Table 5. QX600 Droplet Reader and Included Components** **Component** / **Description** / **Catalog Number** QX600 Droplet Reader / Droplet reading instrument / 12013328 USB cable and power cord / USB cable that connects the computer to the instrument and power cord that connects the instrument to the power source **Note:** To obtain cables or power cords, contact Bio-Rad technical support. / Included Droplet reader plate holders (2) / Positions and secures the 96-well plate in the droplet reader plate compartment / 12006834 To ensure you have everything you need to complete runs on the QX600 Droplet Reader and analyze your results, Table 6 identifies additional components and accessories that you can purchase separately. **Table 6. Additional Accessories and Consumables** **Item** / **Description** / **Catalog Number** Computer / Computer that connects to the QX600 Droplet Reader for data collection and analysis / 12017458 96-well PCR plates / ddPCR 96-well plates / 12001925 Rainin pipets / 20 µl for sample loading 50 µl for droplet transfer 8-channel, 200 µl for oil / L-20, L8-20 L-50, L8-50 L8-200 CT0479068 at CT0479095

BIO-RAD EX.1009.220

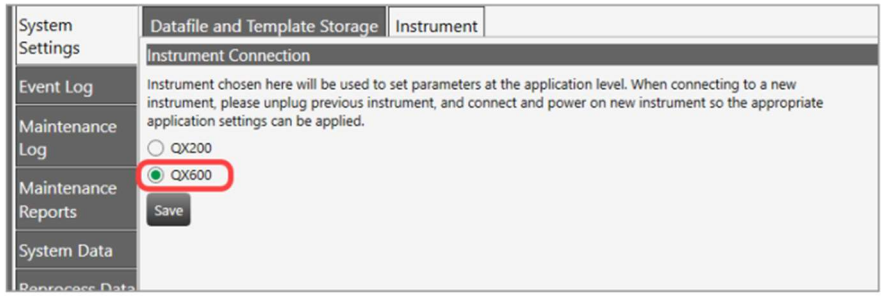
Claims	Exemplary Disclosure of Accused Product
	# Appendix E Ordering Information This appendix contains descriptions and catalog numbers for new or replacement instruments, accessories, and consumables for Bio-Rad's ddPCR products, including your QX600 Droplet Reader. **ddPCR System and Instrument Packages** Table 41 contains information on Bio-Rad Laboratories, Inc. Droplet Digital PCR systems, as well as individual ddPCR instruments and included accessories. **Table 41. ddPCR System and Instrument Packages** | Product	Description	Catalog Number | |---|---|---| | QX200 Droplet Digital PCR System | Includes the QX200 Droplet Generator, QX200 Droplet Reader, computer, software, and consumables | 1864001 | | QX600 Droplet Digital PCR System | Includes the QX200 Droplet Generator, QX600 Droplet Reader, computer, software, and consumables | 17007769 | | QX600 AutoDG™ Droplet Digital PCR System | Includes the Automated Droplet Generator (AutoDG), QX600 Droplet Reader, computer, software, and consumables | 17008371 | | QX200 Droplet Generator | Includes the droplet generator (1), cartridges and gaskets (24 each), cartridge holders (2), and power cord (1) | 1864002 | | QX200 Droplet Reader | Includes the droplet reader (1), plate holders (2), USB cable (1), and power cord (1) | 1864003 | | QX600 Droplet Reader | Includes the droplet reader (1), plate holders (2), USB cable (1), and power cord (1) | 12013328 | | Automated Droplet Generator | Includes the automated droplet generator (1), cooling block accessory (1), oil purge reservoir (1), and power cord (1) | 1864101 | | C1000 Thermal Cycler | Thermal cycler with 384-well reaction module | 1851384 | CT0479068 at CT0479276

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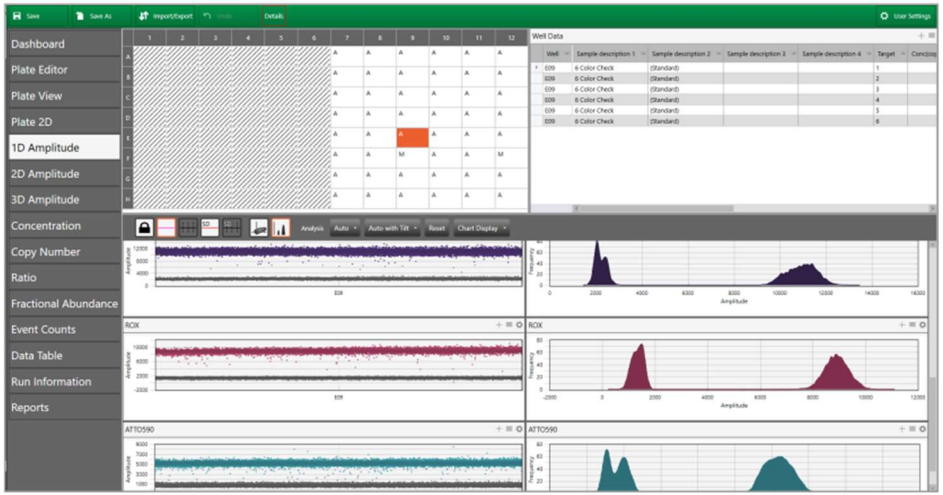
Claims	Exemplary Disclosure of Accused Product
	QX600 Droplet Reader Accessories Table 42 contains information on accessories for the QX600 Droplet Reader. **Table 43. QX600 Droplet Reader Accessories** CT0479068 at CT0479278 QX600's QX Manager Software is run on the attached computer to display the collected fluorophore signal data and conduct analysis on the same. *See, e.g.*:

Product	Description	Catalog Number
Computer	Computer that connects to the QX600 Droplet Reader for data collection and analysis	12017458
USB cable and power cord	Cable connecting the computer to the instrument and power cord connecting the instrument to the power source **Note:** Contact Bio-Rad Technical Support for replacement information.	Included
Droplet reader plate holders (2)	Used to position the 96-well plate in the droplet reader plate compartment	12006834

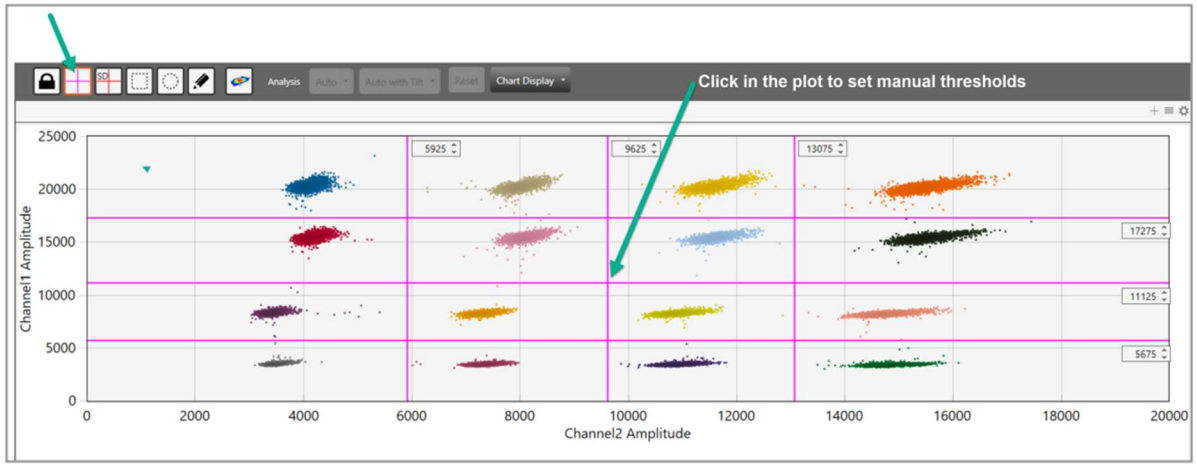
BIO-RAD EX.1009.222

Claims	Exemplary Disclosure of Accused Product
	Selecting the Instrument After First Sign-In When you open the software and sign in for the first time, the instrument connection window appears and you must select the instrument that is connected to the computer. This procedure assumes the instrument is connected to the computer and QX Manager Software is installed. You can use QX Manager Software with the QX200 Droplet Reader and the QX600 Droplet Reader. **To select the instrument** ▶ Select QX600, and then click Save.  **Important:** If your laboratory uses both instruments, you can change instruments at any time. See Changing Instruments on page 165 for information. CT0479068 at CT0479105 The QX Manager Software provides visual plots of first values (e.g., channel intensity values) and second values (e.g., channel wavelength values). *See e.g.*:

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Claims	Exemplary Disclosure of Accused Product
	1D Amplitude Use the 1D Amplitude analysis window to view droplet amplitudes for each channel, in selection order, for each well selected in the Well Selector. The following graphic shows six colors in standard-size droplets.  CT0479068 at CT0479186

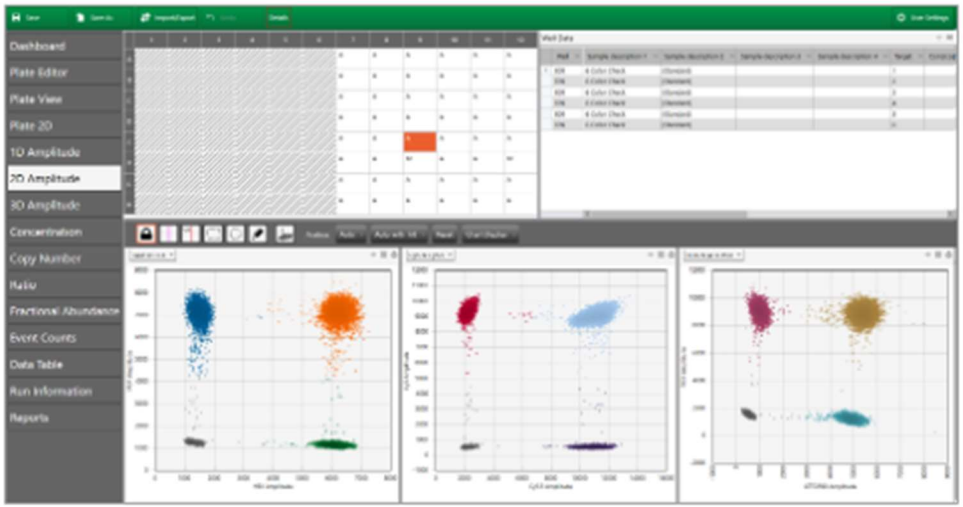
BIO-RAD EX.1009.224

Claims	Exemplary Disclosure of Accused Product
	 CT0479068 at CT0479252 According to at least the examples above, the QX600 comprises a display coupled to the processor (e.g., of a computer connected to the QX600 Droplet Reader running the QX Manager Software), wherein the processor plots, on the display, a representation of the first value (e.g., channel intensity) and the second value (e.g., channel wavelength), both literally and under the doctrine of equivalents.
[19.c.3.A] wherein the cumulative signal data comprises:	The processor processes cumulative signal data, both literally and under the doctrine of equivalents. For example, the QX600 Droplet Reader receives signals from respective droplets through exciting the fluorophores present in the droplets to produce electromagnetic signals, and provides this data to the processor (e.g., of a computer connected to the QX600 Droplet Reader running the QX Manager Software). *See, e.g.*:

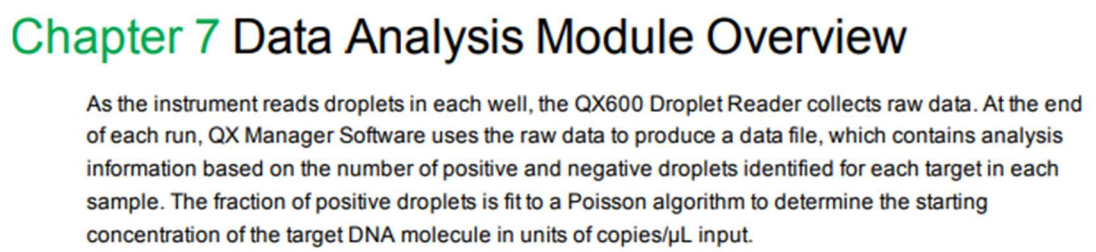
BIO-RAD EX.1009.225

Claims	Exemplary Disclosure of Accused Product
	# Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). CT0479068 at CT0479090 The signals collect fluorescence information about the multiple fluorophores associated with target analytes within droplets, and thus the signal data is cumulative. *See, e.g.*:

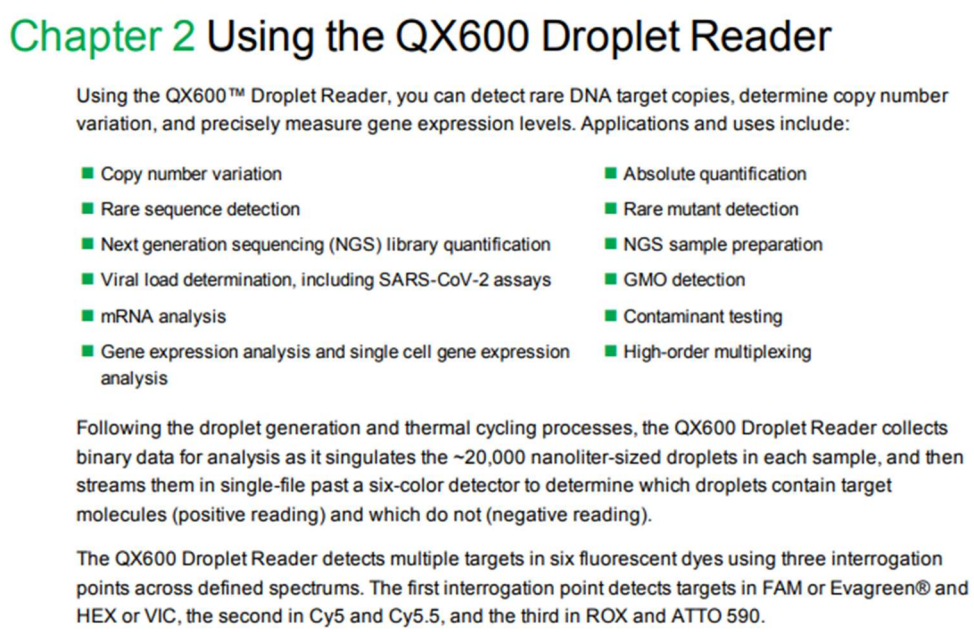
BIO-RAD EX.1009.226

Claims	Exemplary Disclosure of Accused Product
	2D Amplitude For the wells you select in the analysis view of the Plate Editor, the 2D Amplitude window illustrates fluorescence for six targets. The images in this section display data from an experiment involving six targets. Each plot shows individual cluster representations for first and second target positive droplets, droplets containing both targets, and droplets containing neither target. The following graphic shows six colors in standard-size droplets.  CT0479068 at CT0479191 The processor (e.g., of a computer connected to the QX600 Droplet Reader running the QX Manager Software) receives the cumulative signal data and produces a cumulative signal data file containing analysis information based on the number of positive and negative droplets identified for each target in

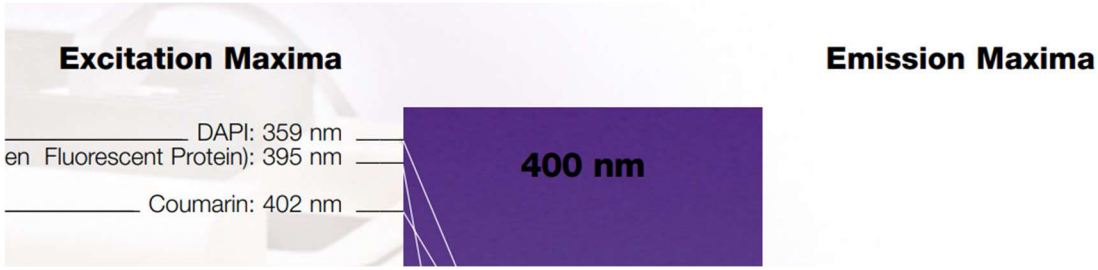
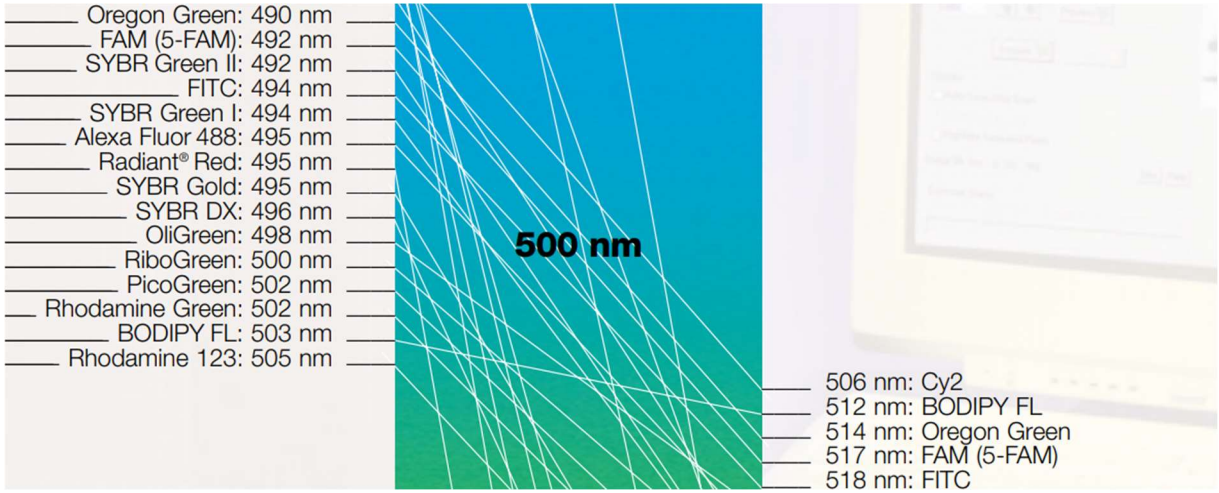
BIO-RAD EX.1009.227

Claims	Exemplary Disclosure of Accused Product
	the sample. *See, e.g.*:  CT0479068 at CT0479144 According to at least the above examples, the processor (e.g., of a computer connected to the QX600 Droplet Reader running the QX Manager Software) processes cumulative signal data, both literally and under the doctrine of equivalents.
[19.c.3.B] a first electromagnetic signal at a first wavelength from the sample volume, the first electromagnetic signal generated by excitement of a first fluorescently	The cumulative signal data comprises a first electromagnetic signal at a first wavelength from the sample volume, the first electromagnetic signal generated by excitement of a first fluorescently labeled polynucleotide analyte of the six fluorescently labeled polynucleotide analytes, both literally and under the doctrine of equivalents. For example, the multi-channel detector (e.g., the QX600 Droplet Reader's six-color detector) detects electromagnetic signals in a FAM fluorophore emission wavelength after interrogating the droplet in a defined spectrum around the FAM fluorophore's excitation wavelength. *See, e.g.*:

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Claims	Exemplary Disclosure of Accused Product
labeled polynucleotide analyte of the six fluorescently labeled polynucleotide analytes;	 CT0479068 at CT0479090 The FAM fluorophore emits a first electromagnetic signal in a FAM fluorophore emission wavelength (e.g., 517 nm), upon excitation (e.g., interrogation) by the QX600 Droplet Reader. *See, e.g.*:

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Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364  CT0479364 at CT0479364

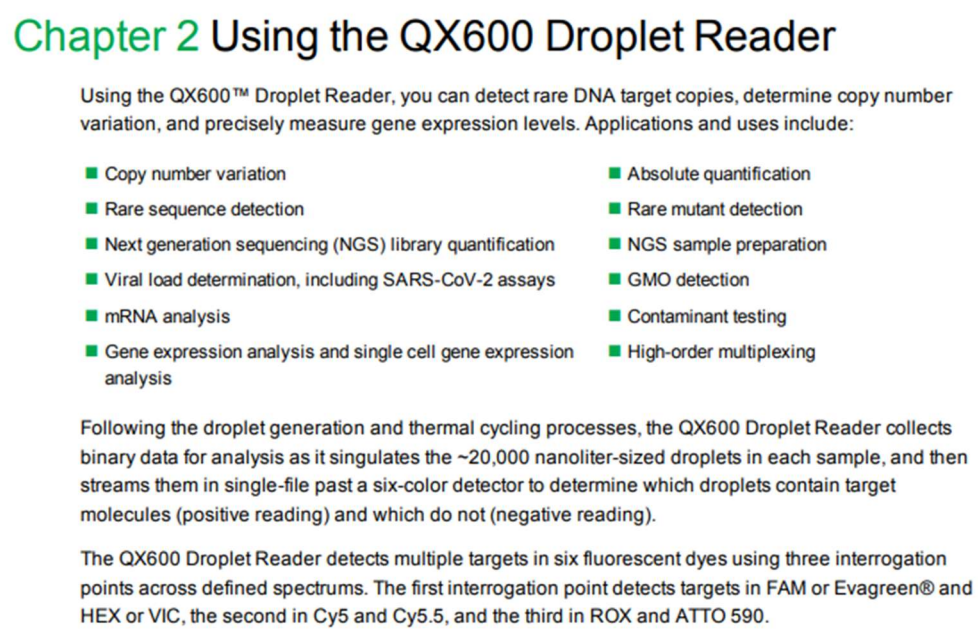
BIO-RAD EX.1009.230

Claims	Exemplary Disclosure of Accused Product
	The FAM fluorophore is one of six fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590) that the QX600 Droplet Reader can detect after excitation (e.g., interrogation) at the given fluorophore excitation wavelength. *See, e.g.*: Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 According to at least the examples above, the cumulative signal data comprises a first electromagnetic signal at a first wavelength (e.g., 517 nm) from the sample chamber (e.g., the 96-well plate), the first electromagnetic signal generated by excitement (e.g., interrogation) of a fluorescently labeled

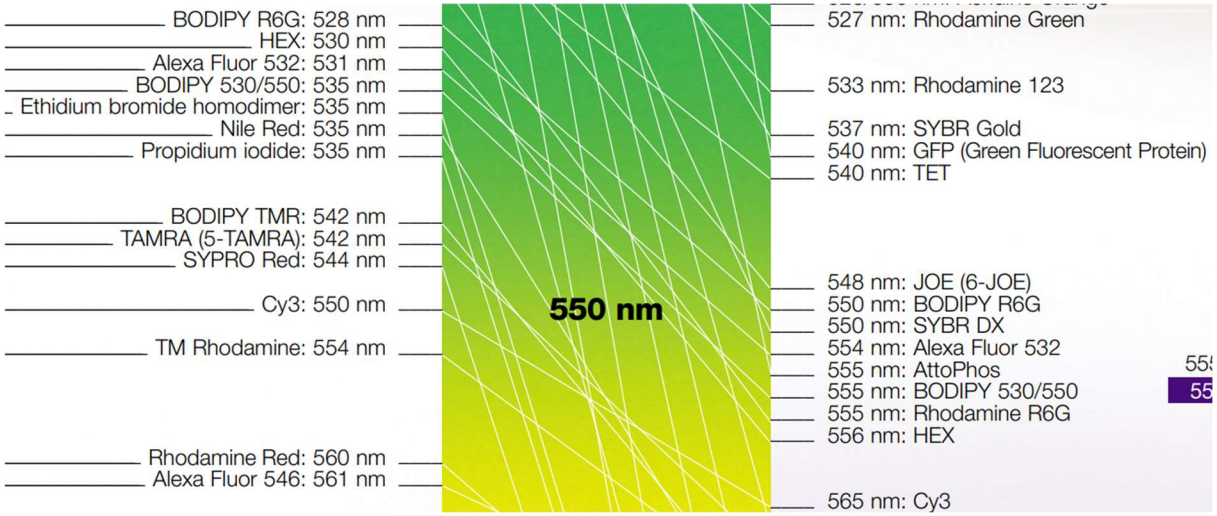
BIO-RAD EX.1009.231

Claims	Exemplary Disclosure of Accused Product
	polynucleotide analyte (e.g., the FAM fluorophore labeled analyte) of the six fluorescently labeled polynucleotide analytes (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590 fluorophore labeled analytes), both literally and under the doctrine of equivalents. While FAM is designated here as the example first fluorophore and its emission wavelength is designated as the first wavelength, the first fluorophore can be any one of the FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590 fluorophores that is not designated as the second, third, or fourth fluorophores, and the first wavelength is the emission wavelength of that fluorophore.
[19.c.3.C] a second electromagnetic signal at a second wavelength from the sample volume, the second electromagnetic signal generated by excitement of a second fluorescently labeled polynucleotide analyte of the six fluorescently labeled polynucleotide analytes;	The cumulative signal data comprises a second electromagnetic signal at a second wavelength from the sample chamber, the second electromagnetic signal generated by excitement of a second fluorescently labeled polynucleotide analyte of the six fluorescently labeled polynucleotide analytes, both literally and under the doctrine of equivalents. For example, the multi-channel detector (e.g., the QX600 Droplet Reader's six-color detector) detects electromagnetic signals in a HEX fluorophore emission wavelength after interrogating the droplet in a defined spectrum around the HEX fluorophore's excitation wavelength. *See, e.g.*:

BIO-RAD EX.1009.232

Claims	Exemplary Disclosure of Accused Product
	 Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 The HEX fluorophore emits a second electromagnetic signal in a HEX fluorophore emission wavelength (e.g., 556 nm), upon excitation (e.g., interrogation) by the QX600 Droplet Reader. *See, e.g.*:

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Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364  CT0479364 at CT0479364

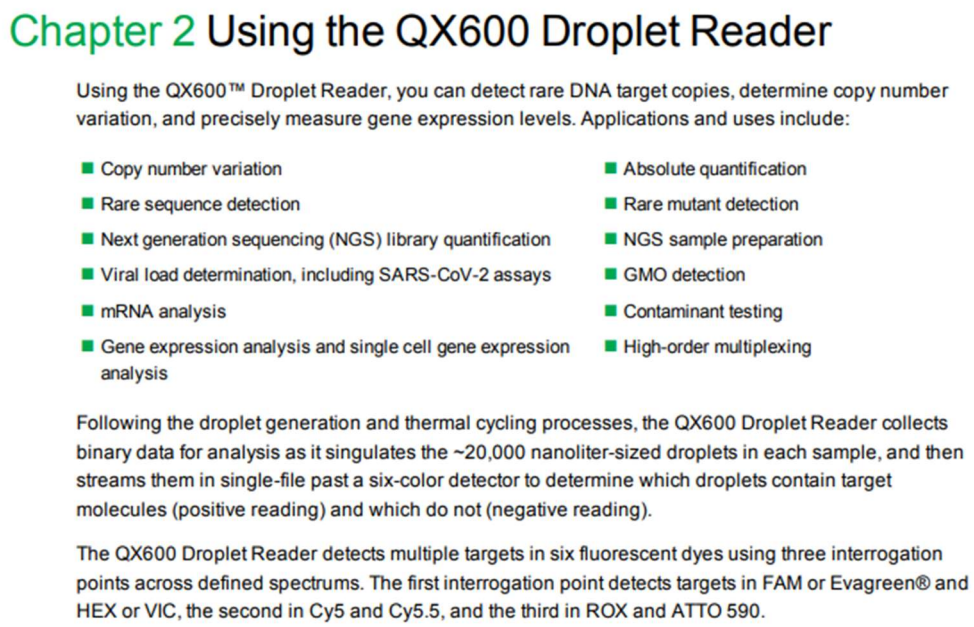
BIO-RAD EX.1009.234

Claims	Exemplary Disclosure of Accused Product
	The HEX fluorophore is one of six fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590) that the QX600 Droplet Reader can detect after excitation (e.g., interrogation) at the given fluorophore excitation wavelength. *See, e.g.*: Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 According to at least the examples above, the cumulative signal data comprises a second electromagnetic signal at a second wavelength (e.g., 556 nm) from the sample chamber (e.g., the 96-well plate), the second electromagnetic signal generated by excitement (e.g., interrogation) of a second fluorescently labeled polynucleotide analyte (e.g., the HEX fluorophore labelled analyte) of the six fluorescently labeled polynucleotide analytes(e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590 fluorophore labeled analytes), both literally and under the doctrine of equivalents.

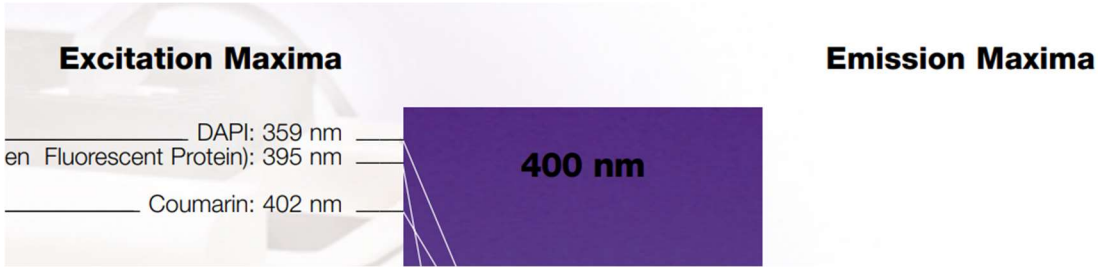
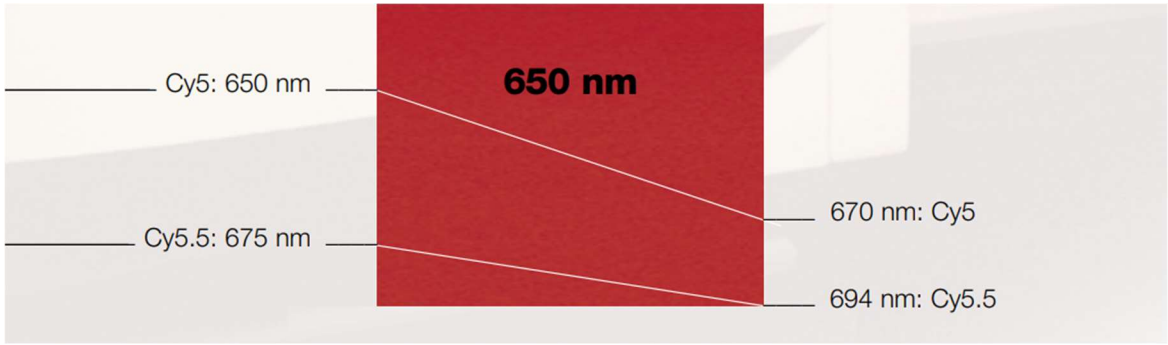
BIO-RAD EX.1009.235

Claims	Exemplary Disclosure of Accused Product
	While HEX is designated here as the example second fluorophore and its emission wavelength is designated as the second wavelength, the second fluorophore can be any one of the FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590 fluorophores that is not designated as the first, third, or fourth fluorophores, and the second wavelength is the emission wavelength of that fluorophore.
[19.c.3.D] a third electromagnetic signal at a third wavelength from the sample volume, the third electromagnetic signal generated by excitement of a third fluorescently labeled polynucleotide analyte of the six fluorescently labeled polynucleotide analytes;	The cumulative signal data comprises a third electromagnetic signal at a third wavelength from the sample chamber, the third electromagnetic signal generated by excitement of a third fluorescently labeled polynucleotide analyte of the six fluorescently labeled polynucleotide analytes, both literally and under the doctrine of equivalents. For example, the multi-channel detector (e.g., the QX600 Droplet Reader's six-color detector) detects electromagnetic signals in a Cy5 fluorophore emission wavelength after interrogating the droplet in a defined spectrum around the Cy5 fluorophore's excitation wavelength. *See, e.g.*:

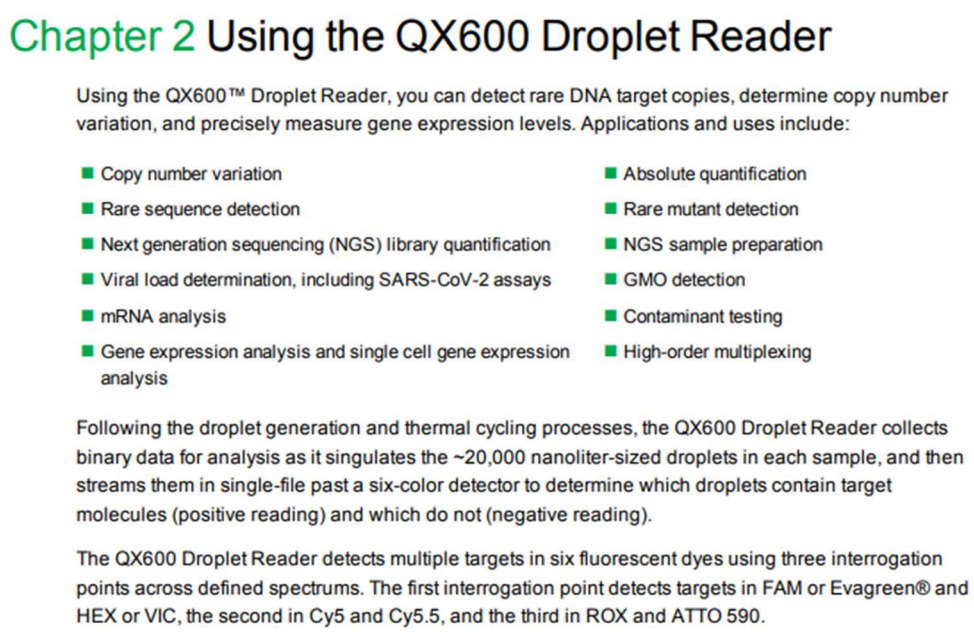
BIO-RAD EX.1009.236

Claims	Exemplary Disclosure of Accused Product
	 Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 The Cy5 fluorophore emits a third electromagnetic signal in a Cy5 fluorophore emission wavelength (e.g., 670 nm), upon excitation (e.g., interrogation) by the QX600 Droplet Reader. *See, e.g.*:

BIO-RAD EX.1009.237

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364  CT0479364 at CT0479364 The Cy5 fluorophore is one of six fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590) that the QX600 Droplet Reader can detect after excitation (e.g., interrogation) at the given fluorophore excitation wavelength. *See, e.g.*:

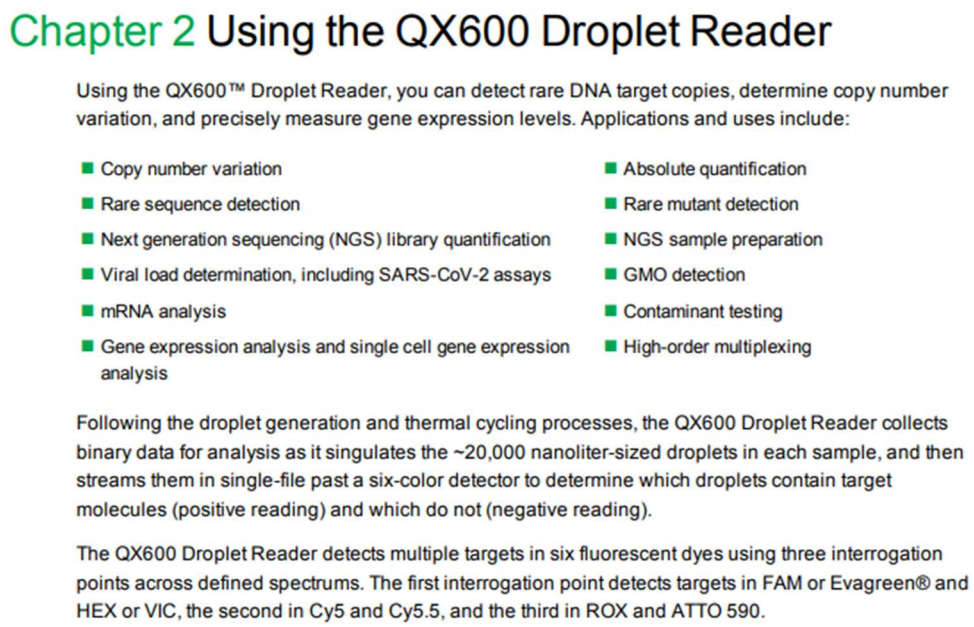
BIO-RAD EX.1009.238

Claims	Exemplary Disclosure of Accused Product
	 Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 According to at least the examples above, the cumulative signal data comprises a third electromagnetic signal at a third wavelength (e.g., 670 nm) from the sample chamber (e.g., the 96-well plate), the third electromagnetic signal generated by excitement (e.g., interrogation) of a third fluorescently labeled polynucleotide analyte (e.g., the Cy5 fluorophore labelled analyte) of the six fluorescently labeled polynucleotide analytes(e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590 fluorophore labeled analytes), both literally and under the doctrine of equivalents. While Cy5 is designated here as the example third fluorophore and its emission wavelength is designated as the third wavelength, the third fluorophore can be any one of the FAM, HEX, Cy5,

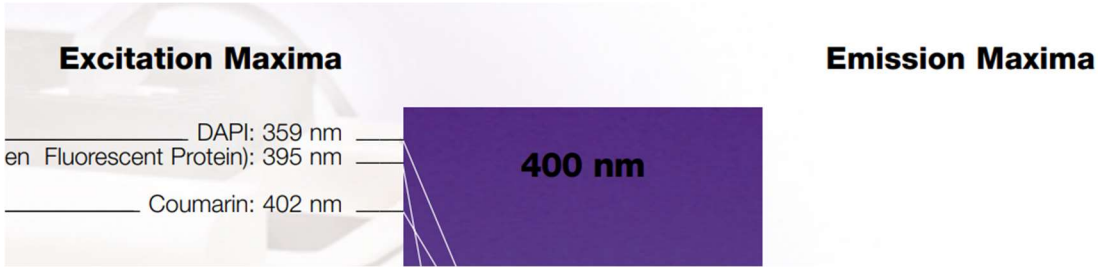
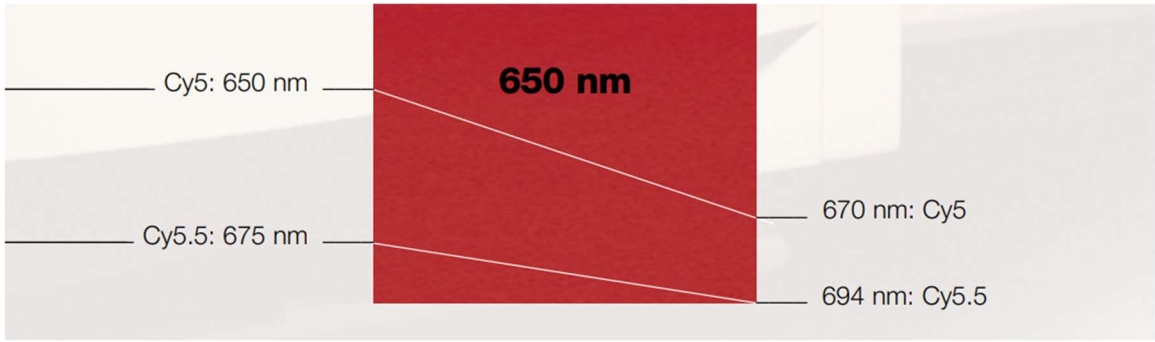
BIO-RAD EX.1009.239

Claims	Exemplary Disclosure of Accused Product
	Cy5.5, ROX, and ATTO 590 fluorophores that is not designated as the first, second, or fourth fluorophores, and the third wavelength is the emission wavelength of that fluorophore.
[19.c.3.E] a fourth electromagnetic signal at a fourth wavelength from the sample volume, the fourth electromagnetic signal generated by excitement of a fourth first fluorescently labeled polynucleotide analyte of the six fluorescently labeled polynucleotide analytes; and	The cumulative signal data comprises a fourth electromagnetic signal at a fourth wavelength from the sample chamber, the fourth electromagnetic signal generated by excitement of a fourth fluorescently labeled polynucleotide analyte of the six fluorescently labeled polynucleotide analytes, both literally and under the doctrine of equivalents. For example, the multi-channel detector (e.g., the QX600 Droplet Reader's six-color detector) detects electromagnetic signals in a Cy5.5 fluorophore emission wavelength after interrogating the droplet in a defined spectrum around the Cy5.5 fluorophore's excitation wavelength. *See, e.g.*:

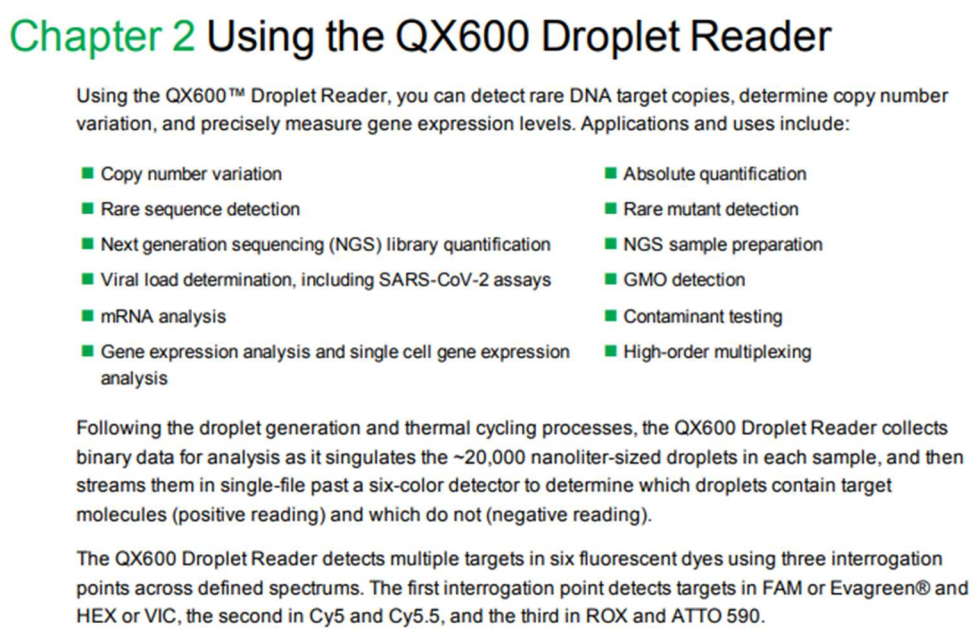
BIO-RAD EX.1009.240

Claims	Exemplary Disclosure of Accused Product
	 Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 The Cy5.5 fluorophore emits a fourth electromagnetic signal in a Cy5.5 fluorophore emission wavelength (e.g., 694 nm), upon excitation (e.g., interrogation) by the QX600 Droplet Reader. *See, e.g.*:

BIO-RAD EX.1009.241

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364  CT0479364 at CT0479364 The Cy5.5 fluorophore is one of six fluorophores (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590) that the QX600 Droplet Reader can detect after excitation (e.g., interrogation) at the given fluorophore excitation wavelength. *See, e.g.*:

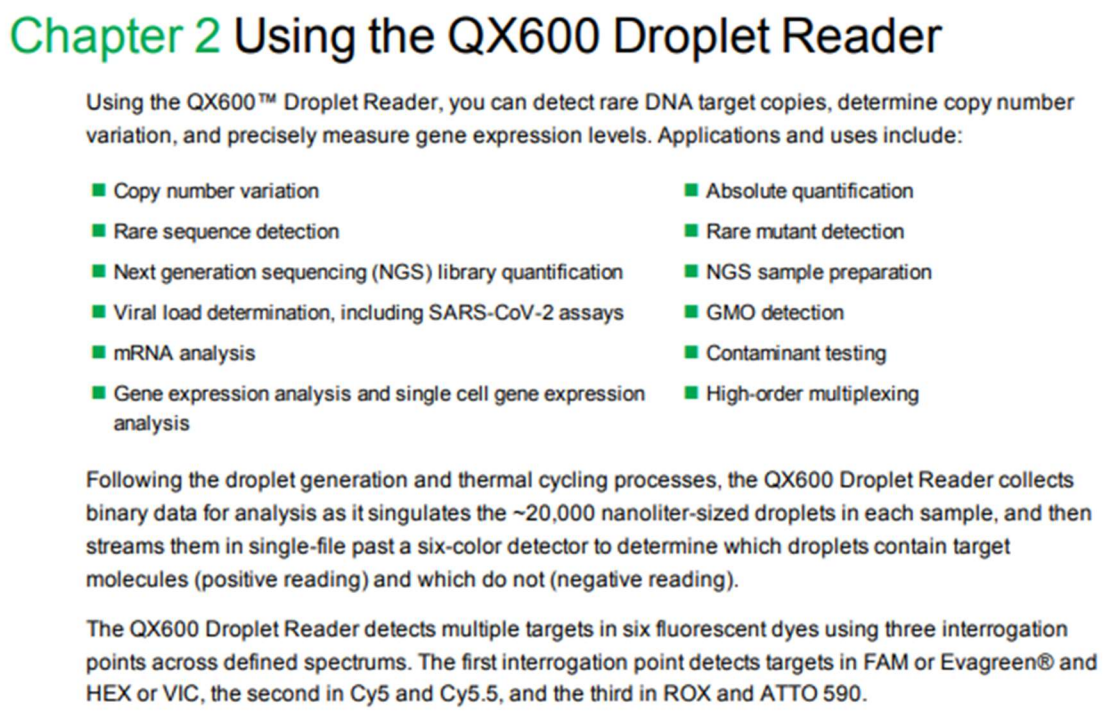
BIO-RAD EX.1009.242

Claims	Exemplary Disclosure of Accused Product
	 Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 According to at least the examples above, the cumulative signal data comprises a fourth electromagnetic signal at a fourth wavelength (e.g., 694 nm) from the sample chamber (e.g., the 96-well plate), the fourth electromagnetic signal generated by excitement (e.g., interrogation) of a fourth fluorescently labeled polynucleotide analyte (e.g., the Cy5.5 fluorophore labelled analyte) of the six fluorescently labeled polynucleotide analytes(e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590 fluorophore labeled analytes), both literally and under the doctrine of equivalents. While Cy5.5 is designated here as the example fourth fluorophore and its emission wavelength is

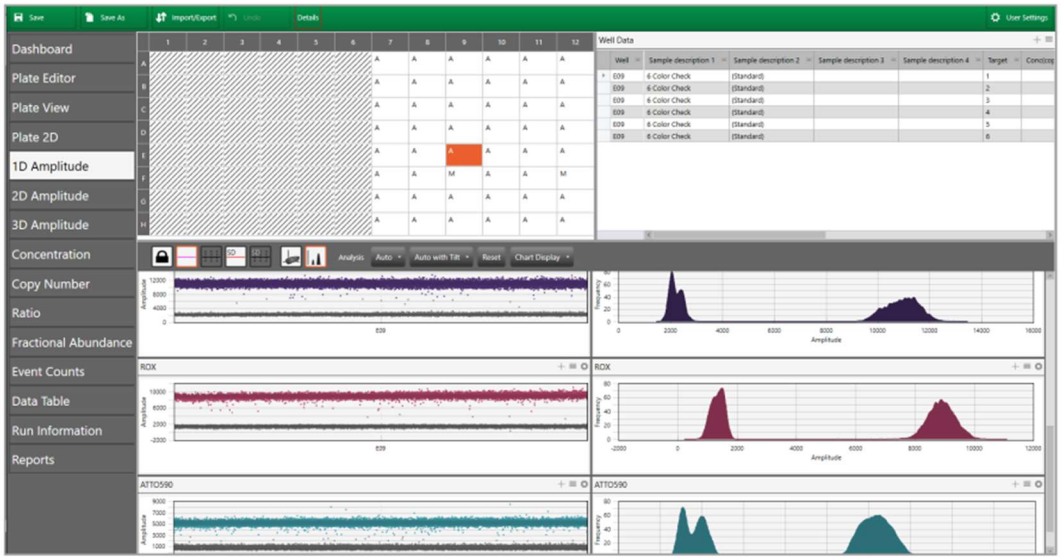
BIO-RAD EX.1009.243

Claims	Exemplary Disclosure of Accused Product
	designated as the fourth wavelength, the fourth fluorophore can be any one of the FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590 fluorophores that is not designated as the first, second, or third fluorophores, and the fourth wavelength is the emission wavelength of that fluorophore.
[19.c.3.F] wherein the second values comprise the first, second, third, and fourth wavelengths, and	The second values of the cumulative signal data comprise the first, second, third, and fourth wavelengths, both literally and under the doctrine of equivalents. The QX600 Droplet Reader collects intensity measurements from three interrogation points covering six fluorophore wavelength channels (e.g., FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590). *See. e.g.*:

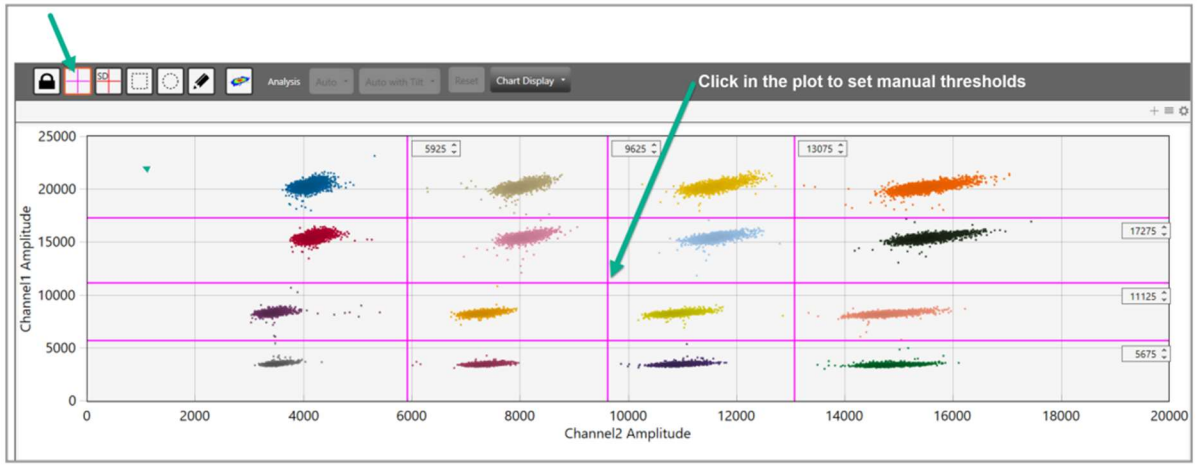
BIO-RAD EX.1009.244

Claims	Exemplary Disclosure of Accused Product
	 Chapter 2 Using the QX600 Droplet Reader Using the QX600™ Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include: ■ Copy number variation ■ Rare sequence detection ■ Next generation sequencing (NGS) library quantification ■ Viral load determination, including SARS-CoV-2 assays ■ mRNA analysis ■ Gene expression analysis and single cell gene expression analysis ■ Absolute quantification ■ Rare mutant detection ■ NGS sample preparation ■ GMO detection ■ Contaminant testing ■ High-order multiplexing Following the droplet generation and thermal cycling processes, the QX600 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a six-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading). The QX600 Droplet Reader detects multiple targets in six fluorescent dyes using three interrogation points across defined spectrums. The first interrogation point detects targets in FAM or Evagreen® and HEX or VIC, the second in Cy5 and Cy5.5, and the third in ROX and ATTO 590. CT0479068 at CT0479090 The QX600 Droplet Reader cumulative signal, includes signal intensity, or amplitude, values for each fluorophore wavelength. *See e.g.*:

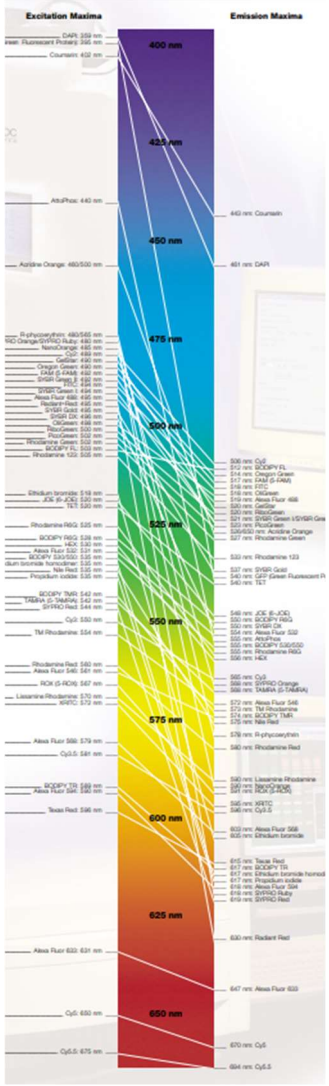
BIO-RAD EX.1009.245

Claims	Exemplary Disclosure of Accused Product
	1D Amplitude Use the 1D Amplitude analysis window to view droplet amplitudes for each channel, in selection order, for each well selected in the Well Selector. The following graphic shows six colors in standard-size droplets.  CT0479068 at CT0479186

BIO-RAD EX.1009.246

Claims	Exemplary Disclosure of Accused Product
	 CT0479068 at CT0479252 Each of the fluorophores has a specific emission wavelength or a range of wavelengths. The emission wavelength for FAM is 517 nm, the emission wavelength for HEX is 556 nm, the emission wavelength for Cy5 is 670 nm, the emission wavelength for Cy5.5 is 694 nm, the emission wavelength for ROX is 591 nm, and the emission wavelength for ATTO 590 is 622 nm. *See, e.g.*:

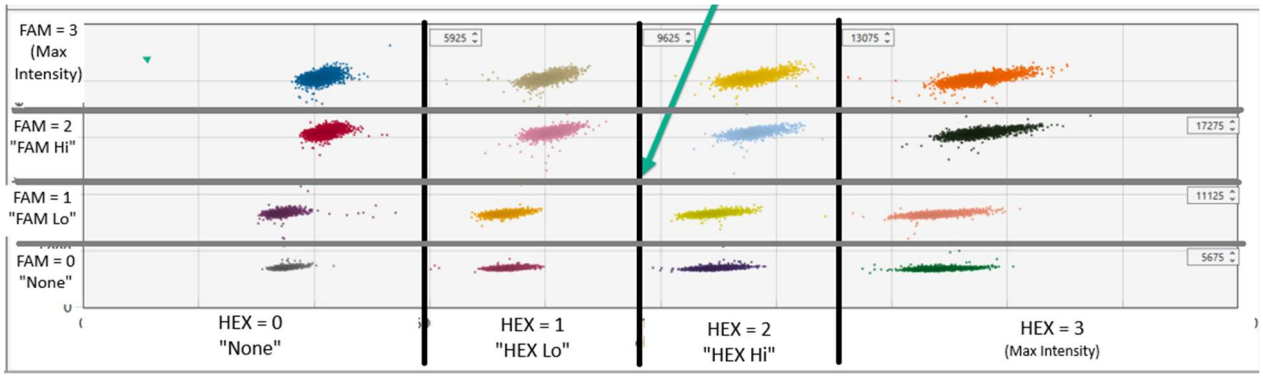
BIO-RAD EX.1009.247

Claims	Exemplary Disclosure of Accused Product
	

BIO-RAD EX.1009.248

Claims	Exemplary Disclosure of Accused Product
	CT0479364 at CT0479364 According to at least the above examples, the second values of the cumulative signal data comprise the first (e.g., FAM wavelength, or 517 nm), second (e.g., HEX wavelength, or 556 nm), third (e.g., Cy5 wavelength, or 670 nm) and fourth (e.g., Cy5.5 wavelength, or 694 nm), both literally and under the doctrine of equivalents.
[19.c.3.G] the determination is made without immobilization, mass spectrometry or melting curve analysis.	Caltech incorporates the discussion regarding above elements of claim 19, *supra,* in their entirety. As discussed in relation to those elements, the unambiguous determination of the presence or absence of at least each of the M analytes in the sample is made without immobilization, mass spectrometry, or melting curve analysis.
[21.pre] 21. The system of claim 19, wherein	Caltech incorporates the discussion from claim 19, *supra,* in its entirety. As discussed therein, the QX600 is a system comprising each and every element of claim 19, both literally and under the doctrine of equivalents.
[21.a] F=3.	The processor makes determinations wherein F=3, both literally and under the doctrine of equivalents. For example, the processor (e.g., of a computer connected to the QX600 Droplet Reader running the QX Manager Software) applies a decoding matrix to the cumulative signal (e.g. assigns target analyte combinations to discrete signal channel levels and associates droplet data with target analyte

BIO-RAD EX.1009.249

Claims	Exemplary Disclosure of Accused Product
	combinations according to signal channel levels within the droplet data), such that maximum cumulative intensity for any channel, F, is three. *See, e.g.*:  CT0479068 at CT0479252 (notation of discretized channel signal intensity levels added, threshold line emphasis added) According at least to the above examples, the processor makes determinations wherein F=3, both literally and under the doctrine of equivalents.

BIO-RAD EX.1009.250

Appendix B: Infringement Claim Chart for U.S. Patent No. 12,168,797 and QX ONE

Bio-Rad's QX ONE Droplet Digital PCR (ddPCR) System ("QX ONE") infringes Claims 1, 5–11, 13–15, and 18 of U.S. Patent No. 12,168,797 ("the '797 Patent"). Reference to the QX ONE will be understood to include all the various additional devices, software, accessories, and/or components used in connection with the QX ONE.

As non-limiting examples, Caltech identifies where and how the QX ONE comprises each and every element of the below claimed systems. Elements from other charts included with these Infringement Contentions may be used in combination with the below to meet elements of the claimed systems, and Caltech incorporates all such other charts here by reference.

On information and belief, the QX ONE is used with devices, accessories, and components to practice claimed systems of the '797 Patent. *See* CT0481971 at CT0481972–CT0481981. A list of non-limiting examples of such devices, accessories, and/or components include:

- The QX ONE system;
- Software, such as the QX ONE Software (Regulatory and/or Standard editions);
- Consumables such as
    - GCR96 Cartridges,
    - QX ONE Droplet Generation Oil for Probes,
    - QX ONE Droplet Reader Oil,
    - GCR96 Foil Heat Seal, and/or
    - QX ONE ddPCR System Waste Bottle;
- A plate sealer, such as the PX1 PCR Plate Sealer;
- ddPCR kits and reagents, such as
    - PrimePCR ddPCR Assays,
    - ddPCR Multiplex Supermix,
    - ddPCR Supermixes for Probes (No dUTP),
    - ddPCR Supermix for Probes,
    - One-Step RT-ddPCR Advanced Kit for Probes,
    - ddPCR Buffer Control for Probes,
    - Residual DNA Quantification Supermixes,
    - QX200 ddPCR EvaGreen Supermix,
    - QX200 Buffer Control for EvaGreen, and
    - ddPCR Library Quantification Kit for Illumina TruSeq;

- Consumables, such as
    - the ddPCR 96-Well Plates,
    - Droplet Generation Oil for Probes,
    - QX200 Droplet Generation Oil for EvaGreen,
    - Droplet Reader Oil,
    - DG8 Cartridges and DG8 Gaskets,
    - DG8 Cartridge Holders,
    - pierceable foil such as the PCR Plate Heat Seal,
    - DG32 Cartridges for AutoDG,
    - Pipet Tips for AutoDG,
    - Pipet Tip Waste Bin for AutoDG;
- PrimePCR Primers, Assays and Arrays;
- Each of the assays identified in these Infringement Contentions; and/or
- Any other devices, software, accessories, and components that Bio-Rad instructs or encourages its customers and end users to use with the QX ONE.

Bio-Rad directly infringes the Asserted Claims by making, using, offering to sell, and/or selling the QX ONE alone and/or with the above-listed devices, software, accessories, and/or components within the United States or by importing the QX ONE alone and/or with the above-listed devices, software, accessories, and/or components to the United States. The QX ONE alone and/or with the above-listed devices, software, accessories, and/or components meets each and every element of the below claimed systems. By way of non-limiting example, Bio-Rad offers to sell the QX ONE in connection with (but not exclusively with) the accompanying QX ONE Software to meet each and every element of the Asserted Claims.

Bio-Rad also indirectly infringes the Asserted Claims by providing the QX ONE alone and/or with the above-listed devices, software, accessories, and/or components. Bio-Rad induces customers and/or end users to infringe by providing the QX ONE to such customers and/or end users, or by providing the QX ONE in combination with the above-listed devices, software, accessories, and/or components to such customers and/or end users, for use in a way that meets every element of the Asserted Claims as described below and throughout these Infringement Contentions in violation of 35 U.S.C. § 271(b). By way of non-limiting example, Bio-Rad provides such customers and/or end users with the QX ONE and the accompanying QX ONE Software for use in a way that meets each and every element of the Asserted Claims.

Bio-Rad also contributes to the infringement of customers and/or end-users by providing the QX ONE to such customers and/or end users, or by providing the QX ONE in combination with the above-listed devices, software, accessories, and/or components to such customers and/or end users, and such customers and/or end-users then use the QX ONE alone and/or with the above-listed devices,

BIO-RAD EX.1009.252

software, accessories, and/or components to meet every element of the Asserted Claims as described below and throughout these Infringement Contentions in violation of 35 U.S.C. § 271(c). By way of non-limiting example, such customers and/or end users use the QX ONE and the accompanying QX ONE Software for use in a way that meets each and every element of the Asserted Claims.

On information and belief, Bio-Rad also supplies or causes to be supplied in or from the United States all or a substantial portion of the QX ONE and/or the above-listed devices, software, accessories, and/or components such that Bio-Rad induces the infringement of customers or end users outside the United States in violation of 35 U.S.C. § 271(f)(1). For like reasons as described above, Bio-Rad contributes to the infringement of customers or end users outside the United States in violation of 35 U.S.C. § 271(f)(2).

Caltech alleges direct and indirect infringement both literally and under the doctrine of equivalents.

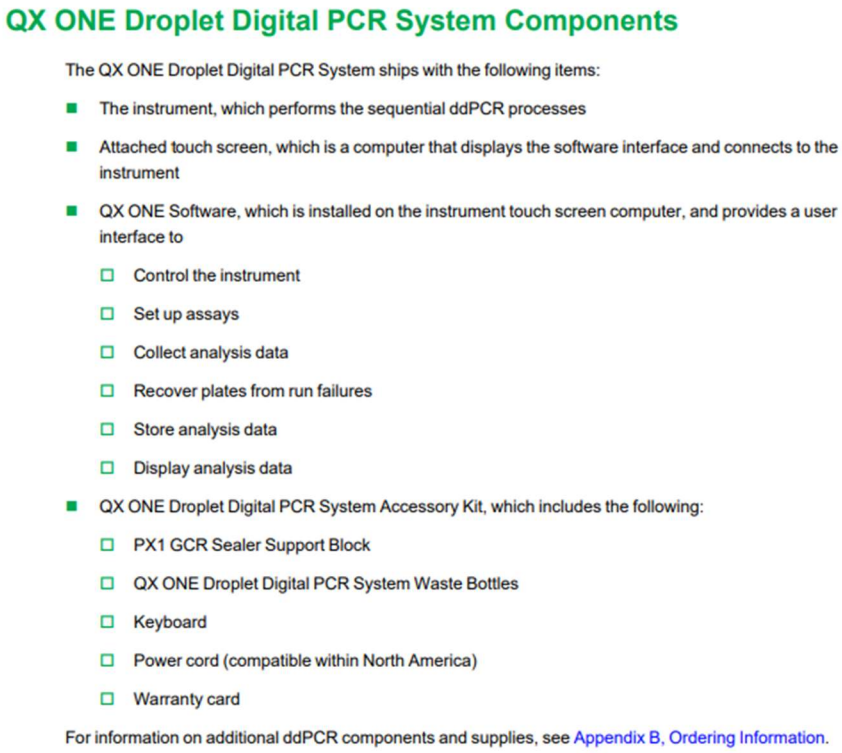
As non-limiting examples, Caltech identifies where and how the QX ONE meets each and every element of the below claimed systems.

Claims	Exemplary Disclosure of Accused Product
[1.pre] 1. A system comprising:	To the extent the preamble is found to be limiting, the QX ONE is a system comprising each and every element below, both literally and under the doctrine of equivalents. For example, the QX ONE is a "system" comprising multiple components, each of which is itself a "system," including at least a droplet generator, droplet reader, thermal cycler, and touchscreen computer for running the QX ONE Software. *See, e.g.*:

BIO-RAD EX.1009.253

Claims	Exemplary Disclosure of Accused Product
	Chapter 2 QX ONE Droplet Digital PCR System The QX ONE™ Droplet Digital PCR System combines droplet generation, thermal cycling, and droplet reading technologies into a single instrument, as described below: ■ The droplet generator partitions samples into approximately 20,000 uniform droplets. ■ The internal thermal cycler performs repeated heating and cooling processes to amplify one or more copies of a particular DNA segment in each droplet. ■ The droplet reader streams the droplets in single file, and then counts the fluorescent positive and negative droplets to calculate and quantify target DNA concentration. The QX ONE Droplet Digital PCR System also features a touch screen computer, which is integrated onto the instrument and has QX ONE Software already installed.  When you open QX ONE Software from the touch screen, the software automatically recognizes the QX ONE Droplet Digital PCR System. **Note:** You cannot directly connect standalone computers to the instrument. However, from a standalone computer you can access files stored on the touch screen computer's internal drive, or from another shared location, as long as all units are connected through the same company network. You can also access the data with a USB drive connected to the touch screen. CT0478736 at CT0478758

BIO-RAD EX.1009.254

Claims	Exemplary Disclosure of Accused Product
	 CT0478736 at CT0478759 According to at least the example provided above, and as explained in further detail for each and every element below, the QX ONE is a system comprising each and every element of independent claim 1, both literally and under the doctrine of equivalents.

BIO-RAD EX.1009.255

Claims	Exemplary Disclosure of Accused Product
[1.a] a sample chamber configured to house a sample and analyte-specific reagent mixtures of analyte-specific hybridization probes and multiple fluorophores;	The QX ONE comprises a sample chamber configured to house a sample and analyte-specific reagent mixtures of analyte-specific hybridization probes and multiple fluorophores, both literally and under the doctrine of equivalents. For example, the QX ONE ddPCR workflow includes combining a sample (e.g., DNA or RNA) with an analyte-specific reagent mixture (e.g., primers, probes, and Bio-Rad ddPCR supermix). *See, e.g.*: **ddPCR Workflow** The ddPCR process adheres to the following workflow: ■ You prepare your samples for PCR by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermix. ■ A droplet generator fractionates a sample into ~20,000 nanoliter-sized droplets with target and background DNA distributed randomly into the droplets during the partitioning process. ■ Following droplet generation, the droplets are run through a thermal cycler, which performs PCR amplification of the nucleic acid target in each individual droplet. ■ A droplet reader reads each droplet to determine the fraction of positive droplets in the original sample and uses Poisson statistical formulas to determine the absolute quantity. **Note:** Positive droplets containing at least one copy of the target DNA molecule exhibit increased fluorescence compared to negative droplets. CT0478736 at CT0478755

BIO-RAD EX.1009.256

Claims	Exemplary Disclosure of Accused Product
	The analyte-specific reagent mixtures (e.g. primers, probes, and Bio-Rad ddPCR supermix) include certain PCR supermixes for probe-based assays, including hydrolysis probe-based assays. *See, e.g.*:

Supermixes

QX ONE Software offers five PCR supermixes. Each supermix is optimized to deliver maximum PCR efficiency and sensitivity for the amplification and detection of DNA and RNA targets.

Important: Use of unapproved supermixes can harm the instrument and void the warranty.

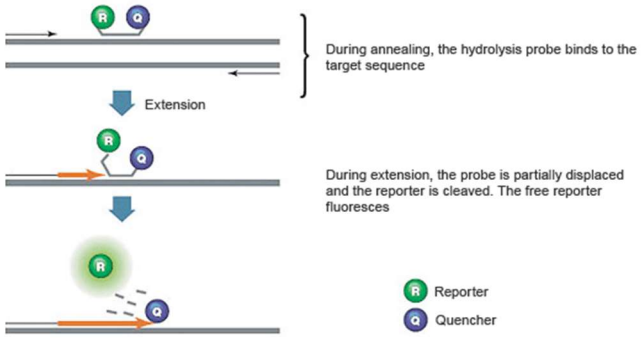
ddPCR Supermix	Description
Multiplex Supermix	For use in nucleic acid sample preparations to amplify and detect DNA targets in probe-based experiments involving multiple targets. **Note:** Bio-Rad recommends using ddPCR Multiplex Supermix for Cy5 and Cy5.5 probes assays.
Supermix for Probes (No dUTP)	For use in nucleic acid sample preparations, with sensitivity for the amplification and detection of DNA targets using hydrolysis probe-based assays.
Supermix for Probes	For use in sample preparation with uracil N-glucosylase (UNG) decontamination protocols to prevent the reamplification of carryover PCR products between experiments.
Supermix for Residual DNA Quantification	For use in residual DNA detection.
One-Step RT Advanced Kit for Probes	For use in absolute quantification of target RNA molecules. **Important:** Load only one (1) one-step RT plate, using the first slot in the QX ONE Droplet Digital PCR System plate Inbox. For more information, see Using the One Step RT ddPCR Advanced Kit for Probes.
EvaGreen® Supermix	For use in gene expression.

CT0478736 at CT0478802

BIO-RAD EX.1009.257

Claims	Exemplary Disclosure of Accused Product
	The analyte-specific reagent mixture includes analyte-specific hybridization probes and multiple fluorophores. Probes in probe-based assays include sequence-specific molecules attached to fluorescent reporter molecules. *See, e.g.*:

BIO-RAD EX.1009.258

Claims	Exemplary Disclosure of Accused Product
	Hydrolysis (TaqMan) Probes Hydrolysis assays (TaqMan or 5' nuclease assays) include a sequence-specific, fluorescently labeled oligonucleotide probe in addition to a sequence-specific PCR primer. Hydrolysis assays exploit the 5' → 3' exonuclease activity of certain thermostable polymerases such as *Taq* or *Tth*. The hydrolysis probe is labeled with a fluorescent reporter at the 5' end and a quencher at the 3' end. Commonly used fluorescent reporter-quencher pairs are fluorescein (FAM), which emits green fluorescence, and Black Hole Quencher 1 dye.  **Fig. 2. Hydrolysis probes are hydrolyzed by *Taq* polymerase.** When the hydrolysis probe is intact, the fluorescence of the reporter is quenched due to its proximity to the quencher (Figure 2). The amplification reaction includes a combined annealing/extension step during which the probe hybridizes to the target, and the dsDNA-specific 5' → 3' exonuclease activity of *Taq* or *Tth* cleaves off the reporter. As a result, the reporter is separated from the quencher, resulting in a fluorescence signal that is proportional to the amount of amplified product in the sample. The main advantages of using hydrolysis probes are high specificity, a high signal-to-noise ratio, and the ability to perform multiplex reactions. The disadvantages are that the initial cost of the probe may be high, and the assay design may not be trivial. CT0481963 at CT0481965–CT0481966

BIO-RAD EX.1009.259

Claims	Exemplary Disclosure of Accused Product
	The analyte-specific reagent mixture is combined with the sample (e.g., DNA or RNA). *See, e.g.*:

Creating the Sample Mix

This section contains a standard protocol using the ddPCR Supermix (No dUTP). You can use this protocol to prepare your samples and set up plates for any assay you perform on the QX ONE Droplet Digital PCR System. The protocol below makes enough reagent for one plate (96 wells). Volumes can be adjusted for the number of plates you need. You can insert up to five plates into the instrument.

Important: You must combine the ddPCR Supermix (No dUTP) and the Reaction Mix in a 1:1 ratio. Final concentrations for components are included in the table below for a total of 20 µl per reaction. For Reaction Mix preparation, refer to each Supermix protocol.

To prepare the sample

1. Remove two ddPCR Supermix (No dUTP) vials of choice and two Reaction Mix vials from storage (-20° C) and let thaw at room temperature for approximately 30 min.
2. Vortex for 10 sec at medium to high speed and spin down in a small tube centrifuge.

   Important: Proper mixing is critical for both the ddPCR Supermix (No dUTP) and the Reaction Mix.
3. Prepare master mix as described in the following table (use 5 ml tubes to prepare mix). Be as accurate as possible in adding the correct volume of each reagent.

Component	Volume, µl	Final Concentration
2x ddPCR Supermix (no dUTP)	1,300	1x
2x Reaction Mix	1,300	1x
TOTAL VOLUME	**2,600**	**1x**

Note: The mixes can be highly viscous. Bio-Rad recommends slow pipeting for more accurate dispensing of all liquid.

a. Carefully pipet 650 µl from each of the two tubes of ddPCR Supermix (No dUTP) into the 5 ml mixing tube, changing tips with each addition.

b. Carefully pipet 650 µl from each of the two tubes of Reaction Mix into the 5 ml mixing tube, changing tips with each addition.

CT0478736 at CT0478783

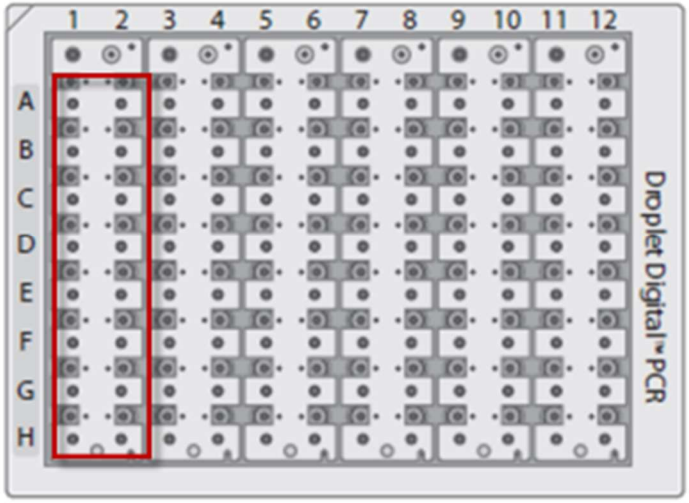
BIO-RAD EX.1009.260

Claims	Exemplary Disclosure of Accused Product
	After combining the sample (e.g., DNA or RNA) analyte (e.g., DNA or RNA) with the analyte-specific reagent mixtures (e.g. primers, probes, and Bio-Rad ddPCR supermix), a droplet generator fractionates the combination into droplets. See e.g.: **ddPCR Workflow** The ddPCR process adheres to the following workflow: ■ You prepare your samples for PCR by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermix. ■ A droplet generator fractionates a sample into ~20,000 nanoliter-sized droplets with target and background DNA distributed randomly into the droplets during the partitioning process. ■ Following droplet generation, the droplets are run through a thermal cycler, which performs PCR amplification of the nucleic acid target in each individual droplet. ■ A droplet reader reads each droplet to determine the fraction of positive droplets in the original sample and uses Poisson statistical formulas to determine the absolute quantity. **Note:** Positive droplets containing at least one copy of the target DNA molecule exhibit increased fluorescence compared to negative droplets. CT0478736 at CT0478755

BIO-RAD EX.1009.261

Claims	Exemplary Disclosure of Accused Product
	Droplet Generation Before droplet generation, ddPCR reactions are prepared in a similar manner as real-time PCR reactions that use TaqMan hydrolysis probes labeled with reporter fluorophores or an intercalating dye. ddPCR is performed with Bio-Rad's proprietary reagents that were developed specifically for droplet generation. The QX ONE™ Droplet Digital PCR System runs the plates containing your DNA samples through the Droplet Generator as the first component in the three-part process. The Droplet Generator uses specially developed reagents and microfluidics to partition each sample into approximately 20,000 nanoliter-sized droplets. Target and background DNA are distributed randomly into the droplets during the partitioning process. Droplet generation produces uniform droplets for the sample, enabling precise target quantification. CT0478736 at CT0478756 The combination of the sample (e.g., DNA or RNA) and the analyte-specific reagent mixture is placed in a GCR96 Cartridge. *See, e.g.*:

BIO-RAD EX.1009.262

Claims	Exemplary Disclosure of Accused Product
	Filling the GCR96 Cartridge You must use GCR96 cartridges with the QX ONE Droplet Digital PCR System. The GCR96 cartridge plate contains 6 DG-16 individual cartridges representing 6 sets of 16 wells. Each set of 16 must be filled with sample or control buffer for the droplet generator to perform correctly. **Important:** GCR cartridges are intended for single-use only. The following graphic highlights the two columns of the first well set in the cartridge.  CT0478736 at CT0478784

BIO-RAD EX.1009.263

Claims	Exemplary Disclosure of Accused Product
	The GCR96 Cartridge containing the droplets is then inserted in the QX ONE instrument for analysis with the QX ONE Software. *See, e.g.*: **Adding Plates in the QX ONE Instrument and Software** The QX ONE Droplet Digital PCR System is equipped with a scanner that is used to scan cartridge (plate) RFID codes. Each GCR96 cartridge has an RFID label embedded on its side, and scanning the code allows the instrument to track the plate and its data.  QX ONE Software prompts you to scan the RFID label on the GCR96 cartridge, and then insert the plate. CT0478736 at CT0478789

BIO-RAD EX.1009.264

Claims	Exemplary Disclosure of Accused Product
	3. Position the plate with the RFID label facing the empty slot with the amber lights, and then insert the plate.  CT0478736 at CT0478791 According to at least the example above, the QX ONE comprises a sample chamber (e.g., the volume within the QX ONE instrument within which the GCR96 Cartridge is inserted) configured to house a sample (e.g., DNA or RNA) and analyte-specific reagent mixtures of analyte-specific hybridization probes and multiple fluorophores (e.g., primers, probes, and Bio-Rad ddPCR supermix mixed together), both literally and under the doctrine of equivalents.

BIO-RAD EX.1009.265

Claims	Exemplary Disclosure of Accused Product
[1.b] a multi-channel detector to detect:	The QX ONE comprises a multi-channel detector, both literally and under the doctrine of equivalents. For example, the QX ONE comprises a multi-channel detector (e.g., an instrument able to detect electromagnetic signals on four channels). *See, e.g.*:

BIO-RAD EX.1009.266

Claims	Exemplary Disclosure of Accused Product
	Instrument Specifications **Table 3. QX ONE Droplet Digital PCR System instrument specifications** **Element** / **Specifications** Size (W x D x H) — 122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor Weight — 100 kg (220 lb) (includes the monitor but not reagents) Capacity — 5 plates Samples — Up to 96 per plate Partitions per sample — Approximately 20,000 droplets Detection channels — 4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel Thermal gradient — Yes Onboard computer — 24 in, 1920 x 1080 resolution Holds data for approximately 100 plates Time to results — 6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol Benchtop requirements — The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. Important: Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis. CT0478736 at CT0478760

BIO-RAD EX.1009.267

Claims	Exemplary Disclosure of Accused Product
	According to at least the example above, the QX ONE comprises a multi-channel detector (e.g., an instrument able to detect electromagnetic signals on four channels), both literally and under the doctrine of equivalents.
[1.b.1] a first electromagnetic signal at a first wavelength from the sample chamber, the first electromagnetic signal generated by excitement of a first fluorophore of the multiple fluorophores;	The multi-channel detector detects a first electromagnetic signal at a first wavelength from the sample chamber, the first electromagnetic signal generated by excitement of a first fluorophore of the multiple fluorophores, both literally and under the doctrine of equivalents. For example, the QX ONE streams the droplets single file in front of the multi-channel detector which measures excited fluorophores as positive or negative droplets to quantify target DNA concentration. *See, e.g.*: Chapter 2 QX ONE Droplet Digital PCR System The QX ONE™ Droplet Digital PCR System combines droplet generation, thermal cycling, and droplet reading technologies into a single instrument, as described below: ■ The droplet generator partitions samples into approximately 20,000 uniform droplets. ■ The internal thermal cycler performs repeated heating and cooling processes to amplify one or more copies of a particular DNA segment in each droplet. ■ The droplet reader streams the droplets in single file, and then counts the fluorescent positive and negative droplets to calculate and quantify target DNA concentration. CT0478736 at CT0478758

BIO-RAD EX.1009.268

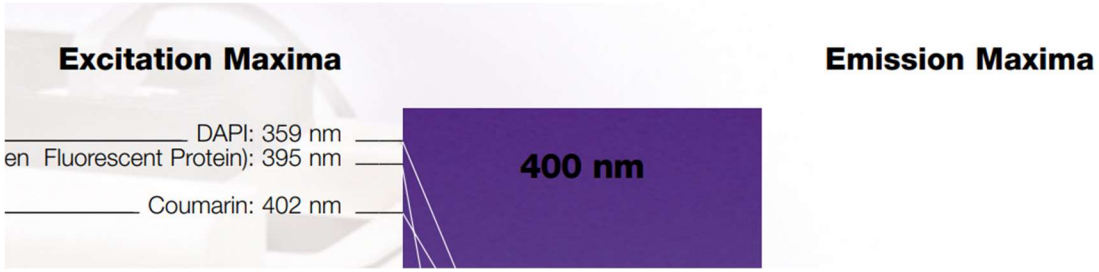
Claims	Exemplary Disclosure of Accused Product
	The multi-channel detector detects electromagnetic signals generated by a first fluorophore (e.g., FAM fluorophores). *See, e.g.*:

Instrument Specifications

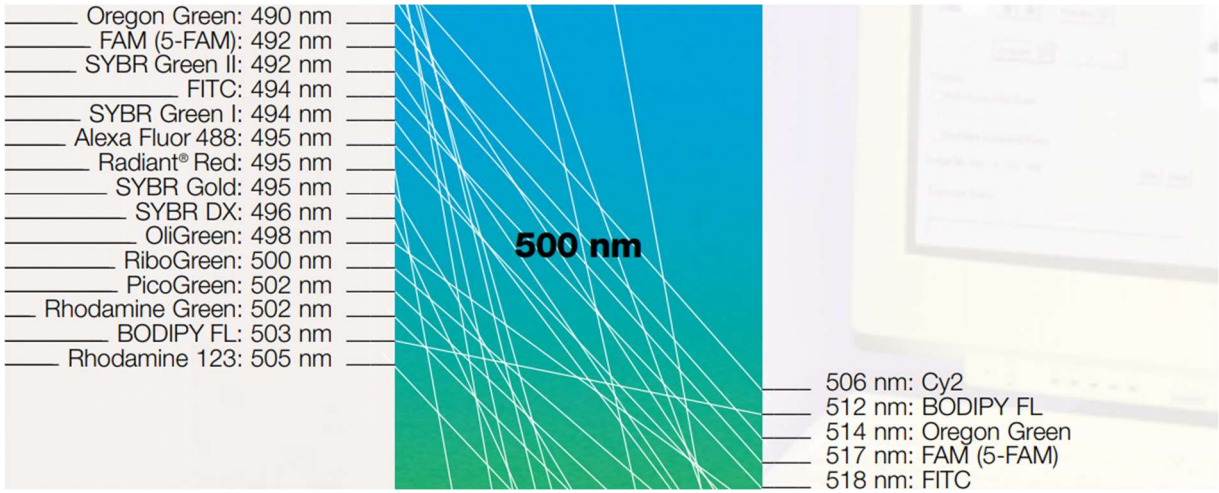
Table 3. QX ONE Droplet Digital PCR System instrument specifications

Element	Specifications
Size (W x D x H)	122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor
Weight	100 kg (220 lb) (includes the monitor but not reagents)
Capacity	5 plates
Samples	Up to 96 per plate
Partitions per sample	Approximately 20,000 droplets
Detection channels	4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel
Thermal gradient	Yes
Onboard computer	24 in, 1920 x 1080 resolution Holds data for approximately 100 plates
Time to results	6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol
Benchtop requirements	The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. **Important:** Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis.

BIO-RAD EX.1009.269

Claims	Exemplary Disclosure of Accused Product
	CT0478736 at CT0478760 The FAM fluorophore emits a first electromagnetic signal in a FAM fluorophore emission wavelength (e.g., 517 nm), upon excitation by the QX ONE. *See, e.g.*:  CT0479364 at CT0479364

BIO-RAD EX.1009.270

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364 The FAM fluorophore is one of multiple fluorophores (e.g., FAM, HEX, Cy5, and Cy5.5) that the QX ONE can detect after excitation at the given fluorophore excitation wavelength. *See, e.g.*:

BIO-RAD EX.1009.271

Claims	Exemplary Disclosure of Accused Product
	Instrument Specifications **Table 3. QX ONE Droplet Digital PCR System instrument specifications** **Element** / **Specifications** Size (W x D x H): 122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor Weight: 100 kg (220 lb) (includes the monitor but not reagents) Capacity: 5 plates Samples: Up to 96 per plate Partitions per sample: Approximately 20,000 droplets Detection channels: 4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel Thermal gradient: Yes Onboard computer: 24 in, 1920 x 1080 resolution Holds data for approximately 100 plates Time to results: 6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol Benchtop requirements: The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. Important: Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis. CT0478736 at CT0478760

BIO-RAD EX.1009.272

Claims	Exemplary Disclosure of Accused Product
	According to at least the example above, the multi-channel detector detects a first electromagnetic signal at a first wavelength (e.g., 517 nm) from the sample chamber, the first electromagnetic signal generated by excitement of a first fluorophore (e.g., the FAM fluorophore) of the multiple fluorophores (e.g., FAM, HEX, Cy5, and Cy5.5), both literally and under the doctrine of equivalents. While FAM is designated here as the example first fluorophore and its emission wavelength is designated as the first wavelength, the first fluorophore can be any one of the FAM, HEX, Cy5, and Cy5.5 fluorophores that is not designated as the second, third, or fourth fluorophores, and the first wavelength is the emission wavelength of that fluorophore.
[1.b.2] a second electromagnetic signal at a second wavelength from the sample chamber, the second electromagnetic signal generated by excitement of a second fluorophore of the multiple fluorophores;	The multi-channel detector detects a second electromagnetic signal at a second wavelength from the sample chamber, the second electromagnetic signal generated by excitement of a second fluorophore of the multiple fluorophores, both literally and under the doctrine of equivalents. For example, the QX One streams the droplets single file in front of the multi-channel detector which measures excited fluorophores as positive or negative droplets to quantify target DNA concentration. *See, e.g.*: Chapter 2 QX ONE Droplet Digital PCR System The QX ONE™ Droplet Digital PCR System combines droplet generation, thermal cycling, and droplet reading technologies into a single instrument, as described below: ■ The droplet generator partitions samples into approximately 20,000 uniform droplets. ■ The internal thermal cycler performs repeated heating and cooling processes to amplify one or more copies of a particular DNA segment in each droplet. ■ The droplet reader streams the droplets in single file, and then counts the fluorescent positive and negative droplets to calculate and quantify target DNA concentration.

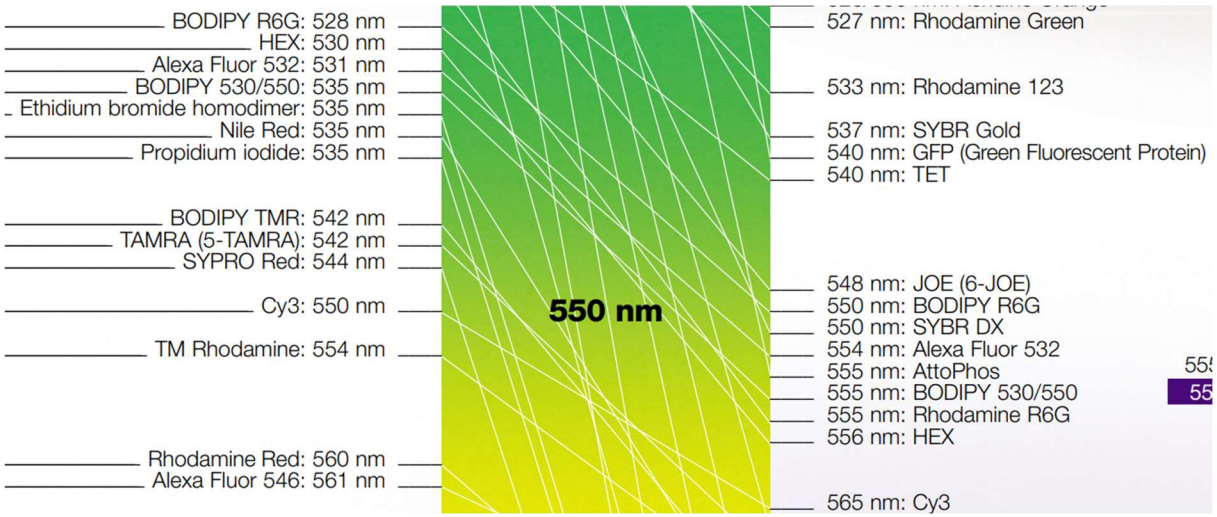
BIO-RAD EX.1009.273

Claims	Exemplary Disclosure of Accused Product
	CT0478736 at CT0478758 The multi-channel detector detects electromagnetic signals generated by a second fluorophore (e.g., HEX fluorophores). *See, e.g.*:

BIO-RAD EX.1009.274

Claims	Exemplary Disclosure of Accused Product
	Instrument Specifications **Table 3. QX ONE Droplet Digital PCR System instrument specifications** **Element** / **Specifications** Size (W x D x H) — 122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor Weight — 100 kg (220 lb) (includes the monitor but not reagents) Capacity — 5 plates Samples — Up to 96 per plate Partitions per sample — Approximately 20,000 droplets Detection channels — 4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel Thermal gradient — Yes Onboard computer — 24 in, 1920 x 1080 resolution Holds data for approximately 100 plates Time to results — 6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol Benchtop requirements — The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. Important: Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis. CT0478736 at CT0478760

BIO-RAD EX.1009.275

Claims	Exemplary Disclosure of Accused Product
	The HEX fluorophore emits a second electromagnetic signal in a HEX fluorophore emission wavelength (e.g., 556 nm), upon excitation by the QX ONE. *See, e.g.*:  CT0479364 at CT0479364  CT0479364 at CT0479364

BIO-RAD EX.1009.276

Claims	Exemplary Disclosure of Accused Product
	The HEX fluorophore is one of multiple fluorophores (e.g., FAM, HEX, Cy5, and Cy5.5) that the QX ONE can detect after excitation at the given fluorophore excitation wavelength. *See, e.g.*:

Instrument Specifications

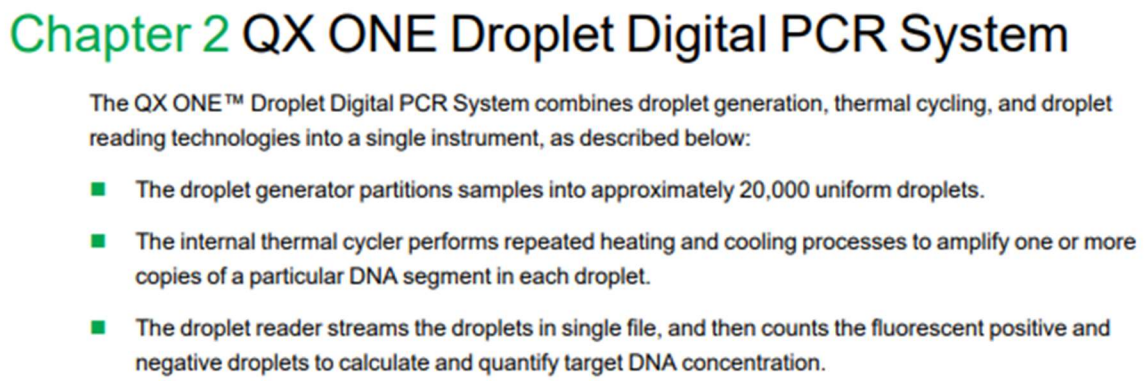
Table 3. QX ONE Droplet Digital PCR System instrument specifications

Element	Specifications
Size (W x D x H)	122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor
Weight	100 kg (220 lb) (includes the monitor but not reagents)
Capacity	5 plates
Samples	Up to 96 per plate
Partitions per sample	Approximately 20,000 droplets
Detection channels	4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel
Thermal gradient	Yes
Onboard computer	24 in, 1920 x 1080 resolution Holds data for approximately 100 plates
Time to results	6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol
Benchtop requirements	The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. **Important:** Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis.

BIO-RAD EX.1009.277

Claims	Exemplary Disclosure of Accused Product
	CT0478736 at CT0478760 According to at least the example above, the multi-channel detector detects a second electromagnetic signal at a second wavelength (e.g., 556 nm) from the sample chamber, the second electromagnetic signal generated by excitement of a second fluorophore (e.g., the HEX fluorophore) of the multiple fluorophores (e.g., FAM, HEX, Cy5, and Cy5.5), both literally and under the doctrine of equivalents. While HEX is designated here as the example second fluorophore and its emission wavelength is designated as the second wavelength, the second fluorophore can be any one of the FAM, HEX, Cy5, and Cy5.5 fluorophores that is not designated as the first, third, or fourth fluorophores, and the second wavelength is the emission wavelength of that fluorophore.
[1.b.3] a third electromagnetic signal at a third wavelength from the sample chamber, the third electromagnetic signal generated by excitement of a third fluorophore of the multiple fluorophores;	The multi-channel detector detects a third electromagnetic signal at a third wavelength from the sample chamber, the third electromagnetic signal generated by excitement of a third fluorophore of the multiple fluorophores, both literally and under the doctrine of equivalents. For example, the QX One streams the droplets single file in front of the multi-channel detector which measures excited fluorophores as positive or negative droplets to quantify target DNA concentration. *See, e.g.*:

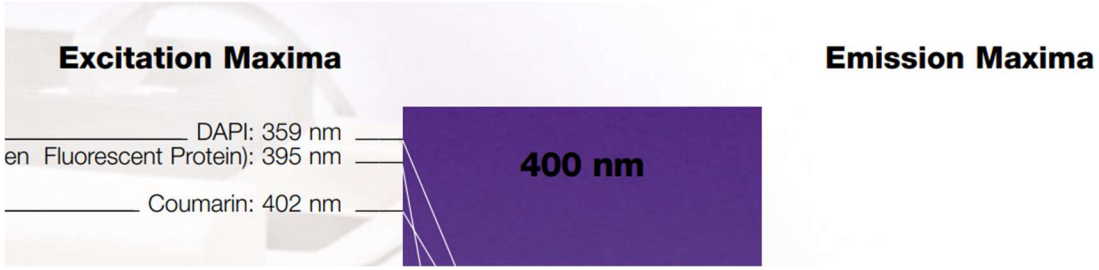
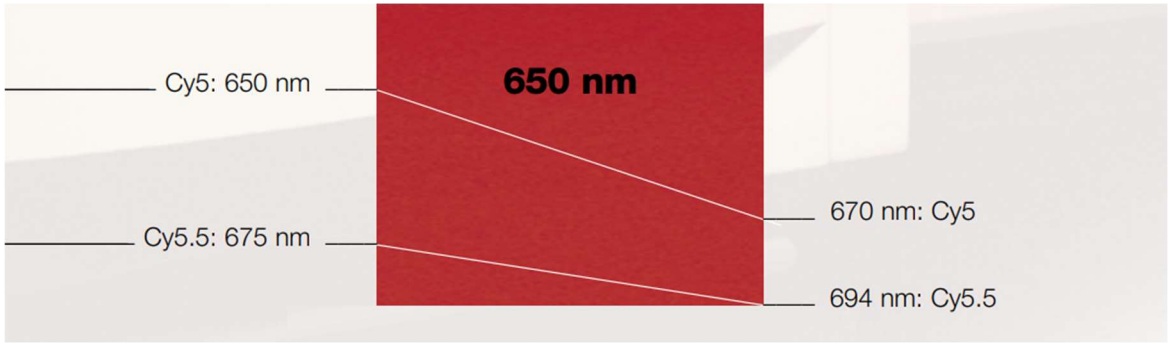
BIO-RAD EX.1009.278

Claims	Exemplary Disclosure of Accused Product
	 CT0478736 at CT0478758 The multi-channel detector detects electromagnetic signals generated by a third fluorophore (e.g., Cy5 fluorophores). *See, e.g.*:

BIO-RAD EX.1009.279

Claims	Exemplary Disclosure of Accused Product
	Instrument Specifications **Table 3. QX ONE Droplet Digital PCR System instrument specifications** **Element** / **Specifications** Size (W x D x H): 122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor Weight: 100 kg (220 lb) (includes the monitor but not reagents) Capacity: 5 plates Samples: Up to 96 per plate Partitions per sample: Approximately 20,000 droplets Detection channels: 4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel Thermal gradient: Yes Onboard computer: 24 in, 1920 x 1080 resolution Holds data for approximately 100 plates Time to results: 6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol Benchtop requirements: The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. Important: Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis. CT0478736 at CT0478760

BIO-RAD EX.1009.280

Claims	Exemplary Disclosure of Accused Product
	The Cy5 fluorophore emits a third electromagnetic signal in a Cy5 fluorophore emission wavelength (e.g., 670 nm), upon excitation by the QX ONE. *See, e.g.*:  CT0479364 at CT0479364  CT0479364 at CT0479364 The Cy5 fluorophore is one of multiple fluorophores (e.g., FAM, HEX, Cy5, and Cy5.5) that the QX ONE can detect after excitation at the given fluorophore excitation wavelength. *See, e.g.*:

BIO-RAD EX.1009.281

Claims	Exemplary Disclosure of Accused Product
	Instrument Specifications **Table 3. QX ONE Droplet Digital PCR System instrument specifications** **Element** / **Specifications** Size (W x D x H) — 122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor Weight — 100 kg (220 lb) (includes the monitor but not reagents) Capacity — 5 plates Samples — Up to 96 per plate Partitions per sample — Approximately 20,000 droplets Detection channels — 4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel Thermal gradient — Yes Onboard computer — 24 in, 1920 x 1080 resolution Holds data for approximately 100 plates Time to results — 6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol Benchtop requirements — The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. Important: Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis. CT0478736 at CT0478760

BIO-RAD EX.1009.282

Claims	Exemplary Disclosure of Accused Product
	According to at least the example above, the multi-channel detector detects a third electromagnetic signal at a third wavelength (e.g., 670 nm) from the sample chamber, the third electromagnetic signal generated by excitement of a third fluorophore (e.g., the Cy5 fluorophore) of the multiple fluorophores (e.g., FAM, HEX, Cy5, and Cy5.5), both literally and under the doctrine of equivalents. While Cy5 is designated here as the example third fluorophore and its emission wavelength is designated as the third wavelength, the third fluorophore can be any one of the FAM, HEX, Cy5, and Cy5.5 fluorophores that is not designated as the first, second, or fourth fluorophores, and the third wavelength is the emission wavelength of that fluorophore.
[1.b.4] a fourth electromagnetic signal at a fourth wavelength from the sample chamber, the fourth electromagnetic signal generated by excitement of a fourth fluorophore of the multiple fluorophores;	The multi-channel detector detects a fourth electromagnetic signal at a fourth wavelength from the sample chamber, the fourth electromagnetic signal generated by excitement of a fourth fluorophore of the multiple fluorophores, both literally and under the doctrine of equivalents. For example, the QX ONE streams the droplets single file in front of the multi-channel detector which measures excited fluorophores as positive or negative droplets to quantify target DNA concentration. *See, e.g.*: Chapter 2 QX ONE Droplet Digital PCR System The QX ONE™ Droplet Digital PCR System combines droplet generation, thermal cycling, and droplet reading technologies into a single instrument, as described below: ■ The droplet generator partitions samples into approximately 20,000 uniform droplets. ■ The internal thermal cycler performs repeated heating and cooling processes to amplify one or more copies of a particular DNA segment in each droplet. ■ The droplet reader streams the droplets in single file, and then counts the fluorescent positive and negative droplets to calculate and quantify target DNA concentration.

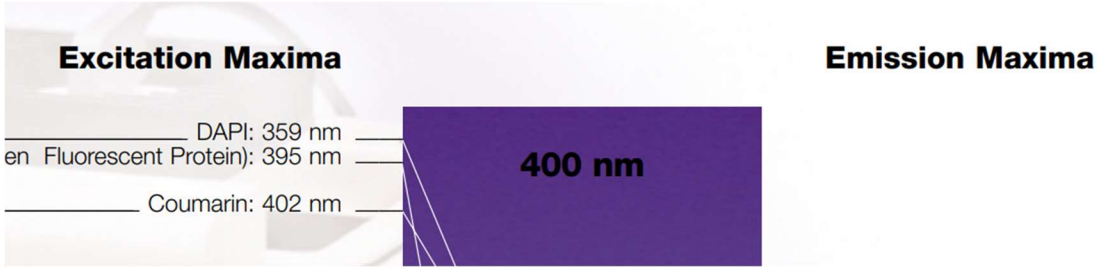
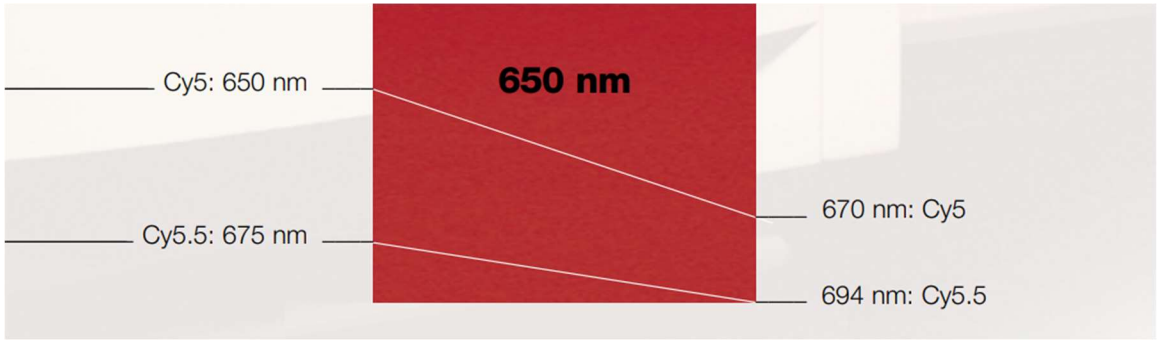
BIO-RAD EX.1009.283

Claims	Exemplary Disclosure of Accused Product
	CT0478736 at CT0478758 The multi-channel detector detects electromagnetic signals generated by a fourth fluorophore (e.g., Cy5.5 fluorophores). *See, e.g.*:

BIO-RAD EX.1009.284

Claims	Exemplary Disclosure of Accused Product
	Instrument Specifications **Table 3. QX ONE Droplet Digital PCR System instrument specifications** **Element** / **Specifications** Size (W x D x H): 122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor Weight: 100 kg (220 lb) (includes the monitor but not reagents) Capacity: 5 plates Samples: Up to 96 per plate Partitions per sample: Approximately 20,000 droplets Detection channels: 4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel Thermal gradient: Yes Onboard computer: 24 in, 1920 x 1080 resolution Holds data for approximately 100 plates Time to results: 6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol Benchtop requirements: The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. Important: Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis. CT0478736 at CT0478760

BIO-RAD EX.1009.285

Claims	Exemplary Disclosure of Accused Product
	The Cy5.5 fluorophore emits a fourth electromagnetic signal in a Cy5.5 fluorophore emission wavelength (e.g., 694 nm), upon excitation by the QX ONE. *See, e.g.*:  CT0479364 at CT0479364  CT0479364 at CT0479364 The Cy5.5 fluorophore is one of multiple fluorophores (e.g., FAM, HEX, Cy5, and Cy5.5) that the QX ONE can detect after excitation at the given fluorophore excitation wavelength. *See, e.g.*:

BIO-RAD EX.1009.286

Claims	Exemplary Disclosure of Accused Product
	Instrument Specifications **Table 3. QX ONE Droplet Digital PCR System instrument specifications** CT0478736 at CT0478760

Element	Specifications
Size (W x D x H)	122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor
Weight	100 kg (220 lb) (includes the monitor but not reagents)
Capacity	5 plates
Samples	Up to 96 per plate
Partitions per sample	Approximately 20,000 droplets
Detection channels	4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel
Thermal gradient	Yes
Onboard computer	24 in, 1920 x 1080 resolution Holds data for approximately 100 plates
Time to results	6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol
Benchtop requirements	The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. **Important:** Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis.

BIO-RAD EX.1009.287

Claims	Exemplary Disclosure of Accused Product
	According to at least the example above, the multi-channel detector detects a fourth electromagnetic signal at a fourth wavelength (e.g., 694 nm) from the sample chamber, the fourth electromagnetic signal generated by excitement of a fourth fluorophore (e.g., the Cy5.5 fluorophore) of the multiple fluorophores (e.g., FAM, HEX, Cy5, and Cy5.5), both literally and under the doctrine of equivalents. While Cy5.5 is designated here as the example fourth fluorophore and its emission wavelength is designated as the fourth wavelength, the fourth fluorophore can be any one of the FAM, HEX, Cy5, and Cy5.5 fluorophores that is not designated as the first, second, or third fluorophores, and the fourth wavelength is the emission wavelength of that fluorophore.
[1.c] a processor controlled analyzer to	The QX ONE comprises a processor controlled analyzer, both literally and under the doctrine of equivalents. For example, the QX ONE comprises a touch screen computer, which is a processor controlled analyzer and which is integrated into the QX ONE instrument. Users may also alternatively connect a standalone computer, also a processor controlled analyzer, to the QX ONE instrument via a network connection *See, e.g.*:

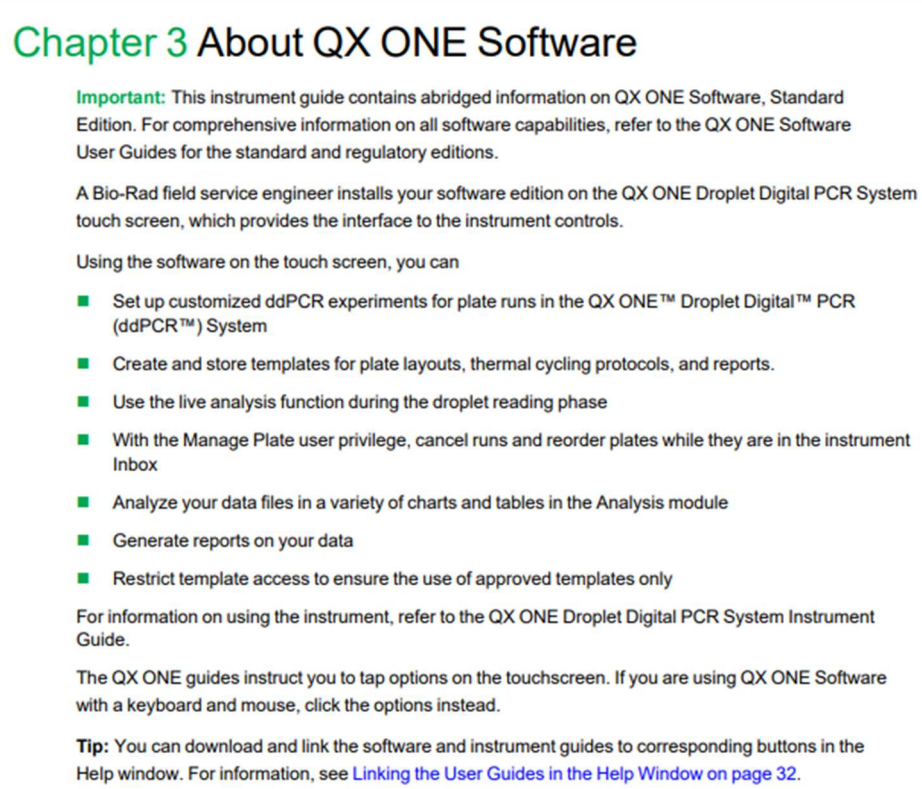
BIO-RAD EX.1009.288

Claims	Exemplary Disclosure of Accused Product
	Chapter 2 QX ONE Droplet Digital PCR System The QX ONE™ Droplet Digital PCR System combines droplet generation, thermal cycling, and droplet reading technologies into a single instrument, as described below: ■ The droplet generator partitions samples into approximately 20,000 uniform droplets. ■ The internal thermal cycler performs repeated heating and cooling processes to amplify one or more copies of a particular DNA segment in each droplet. ■ The droplet reader streams the droplets in single file, and then counts the fluorescent positive and negative droplets to calculate and quantify target DNA concentration. The QX ONE Droplet Digital PCR System also features a touch screen computer, which is integrated onto the instrument and has QX ONE Software already installed.  When you open QX ONE Software from the touch screen, the software automatically recognizes the QX ONE Droplet Digital PCR System. **Note:** You cannot directly connect standalone computers to the instrument. However, from a standalone computer you can access files stored on the touch screen computer's internal drive, or from another shared location, as long as all units are connected through the same company network. You can also access the data with a USB drive connected to the touch screen. CT0478736 at CT0478758

BIO-RAD EX.1009.289

Claims	Exemplary Disclosure of Accused Product
	QX ONE Droplet Digital PCR System Components The QX ONE Droplet Digital PCR System ships with the following items: ■ The instrument, which performs the sequential ddPCR processes ■ Attached touch screen, which is a computer that displays the software interface and connects to the instrument ■ QX ONE Software, which is installed on the instrument touch screen computer, and provides a user interface to □ Control the instrument □ Set up assays □ Collect analysis data □ Recover plates from run failures □ Store analysis data □ Display analysis data ■ QX ONE Droplet Digital PCR System Accessory Kit, which includes the following: □ PX1 GCR Sealer Support Block □ QX ONE Droplet Digital PCR System Waste Bottles □ Keyboard □ Power cord (compatible within North America) □ Warranty card For information on additional ddPCR components and supplies, see Appendix B, Ordering Information. CT0478736 at CT0478759 The touch screen or standalone computer runs the QX ONE Software to analyze sample data. *See, e.g.*:

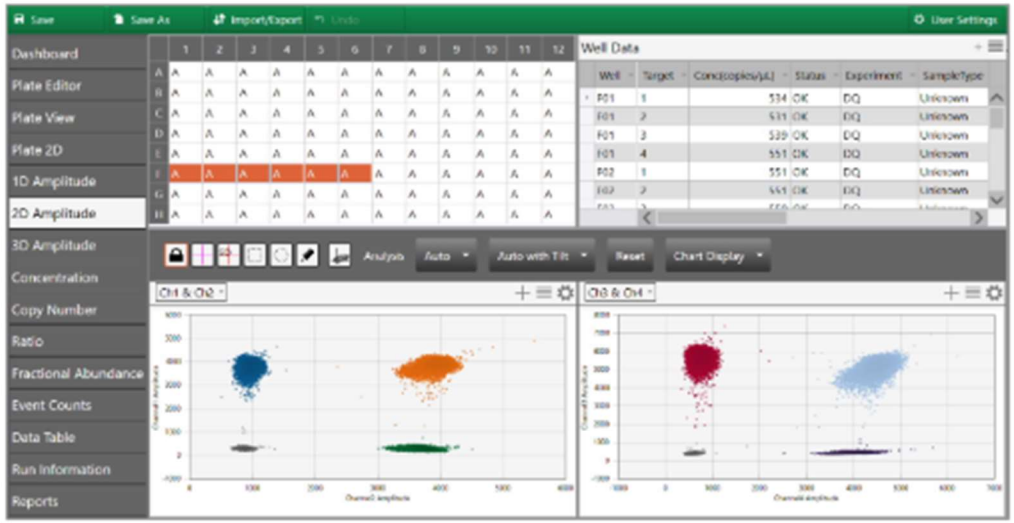
BIO-RAD EX.1009.290

Claims	Exemplary Disclosure of Accused Product
	 Chapter 3 About QX ONE Software Important: This instrument guide contains abridged information on QX ONE Software, Standard Edition. For comprehensive information on all software capabilities, refer to the QX ONE Software User Guides for the standard and regulatory editions. A Bio-Rad field service engineer installs your software edition on the QX ONE Droplet Digital PCR System touch screen, which provides the interface to the instrument controls. Using the software on the touch screen, you can ■ Set up customized ddPCR experiments for plate runs in the QX ONE™ Droplet Digital™ PCR (ddPCR™) System ■ Create and store templates for plate layouts, thermal cycling protocols, and reports. ■ Use the live analysis function during the droplet reading phase ■ With the Manage Plate user privilege, cancel runs and reorder plates while they are in the instrument Inbox ■ Analyze your data files in a variety of charts and tables in the Analysis module ■ Generate reports on your data ■ Restrict template access to ensure the use of approved templates only For information on using the instrument, refer to the QX ONE Droplet Digital PCR System Instrument Guide. The QX ONE guides instruct you to tap options on the touchscreen. If you are using QX ONE Software with a keyboard and mouse, click the options instead. **Tip:** You can download and link the software and instrument guides to corresponding buttons in the Help window. For information, see Linking the User Guides in the Help Window on page 32. CT0478736 at CT0478766 According to at least the example above, the QX ONE comprises a processor controlled analyzer (e.g. an integrated touch screen computer or standalone computer running the QX ONE software), both literally and under the doctrine of equivalents.

BIO-RAD EX.1009.291

Claims	Exemplary Disclosure of Accused Product
[1.c.1] receive, from the multi-channel detector, a cumulative signal based on the first, second, third, and fourth electromagnetic signals and	The processor controlled analyzer receives, from the multi-channel detector a cumulative signal based on the first, second, third, and fourth, electromagnetic signals, both literally and under the doctrine of equivalents. For example, the QX ONE collects receives signals from respective droplets through exciting the fluorophores present in the droplets to produce electromagnetic signals and provides this data to the processor controlled analyzer (e.g. an integrated touch screen computer or standalone computer running the QX ONE software). *See, e.g.*: Chapter 2 QX ONE Droplet Digital PCR System The QX ONE™ Droplet Digital PCR System combines droplet generation, thermal cycling, and droplet reading technologies into a single instrument, as described below: ■ The droplet generator partitions samples into approximately 20,000 uniform droplets. ■ The internal thermal cycler performs repeated heating and cooling processes to amplify one or more copies of a particular DNA segment in each droplet. ■ The droplet reader streams the droplets in single file, and then counts the fluorescent positive and negative droplets to calculate and quantify target DNA concentration. CT0478736 at CT0478758 The signals collect fluorescence information about the multiple fluorophores associated with target analytes within droplets, and thus the signal data is cumulative. *See, e.g.*:

BIO-RAD EX.1009.292

Claims	Exemplary Disclosure of Accused Product
	2D Amplitude For the wells you select in the analysis view of the Plate Editor, the 2D Amplitude window illustrates fluorescence for four targets. The images in this section display data from an experiment involving four targets. Each plot shows individual cluster representations for first and second target positive droplets, droplets containing both targets, and droplets containing neither target.  CT0478876 at CT0478990 The QX ONE software receives the cumulative signal data from the multi-channel detector and produces a data file containing analysis information based on the number of positive and negative droplets identified for each target in the sample. *See, e.g.*:

BIO-RAD EX.1009.293

Claims	Exemplary Disclosure of Accused Product
	# Chapter 3 Data Analysis Module Overview As the instrument reads droplets in each well, the QX ONE Droplet Digital PCR System collects raw data. At the end of each run, QX ONE Software uses the raw data to produce a data file, which contains analysis information based on the number of positive and negative droplets identified for each target in each sample. The fraction of positive droplets is fit to a Poisson algorithm to determine the starting concentration of the target DNA molecule in units of copies/µl input. This section summarizes the analysis functionality, and also describes the general options governing your chart displays. More detailed information, including specific information and uses for each analysis window, as well as information on data calculations, is contained in Chapter 4, Data Analysis Methodology. When you select the Data Analysis tab from the touch screen, you can access the Data Analysis module. When you select the tab from the software installed on a standalone computer, you can also access the Gene Study module.  When you open the data file in the Analysis module, you can view the data with the automatic analysis thresholds and calculations, or you can choose different options to recalculate, replot, or recolor the results. CT0478876 at CT0478942

BIO-RAD EX.1009.294

Claims	Exemplary Disclosure of Accused Product
	The cumulative signal data collected by the QX ONE is based on detections at a first (e.g., FAM emission wavelength), second (e.g., HEX emission wavelength), third (e.g., Cy5 emission wavelength), and fourth (e.g., Cy5.5 emission wavelength) electromagnetic signals. *See, e.g.*:

BIO-RAD EX.1009.295

Instrument Specifications

Table 3. QX ONE Droplet Digital PCR System instrument specifications

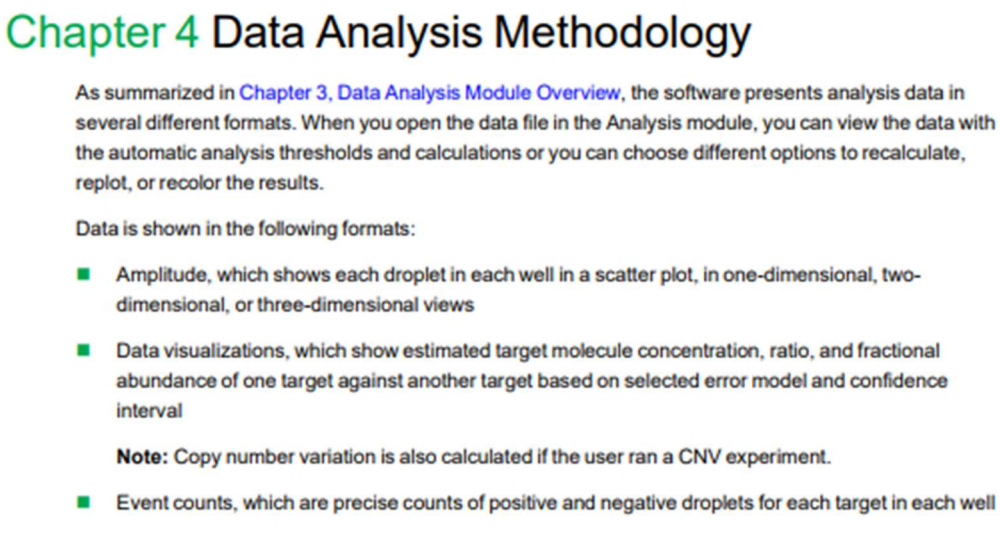
Element	Specifications
Size (W x D x H)	122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor
Weight	100 kg (220 lb) (includes the monitor but not reagents)
Capacity	5 plates
Samples	Up to 96 per plate
Partitions per sample	Approximately 20,000 droplets
Detection channels	4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel
Thermal gradient	Yes
Onboard computer	24 in, 1920 x 1080 resolution Holds data for approximately 100 plates
Time to results	6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol
Benchtop requirements	The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. **Important:** Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis.

CT0478736 at CT0478760

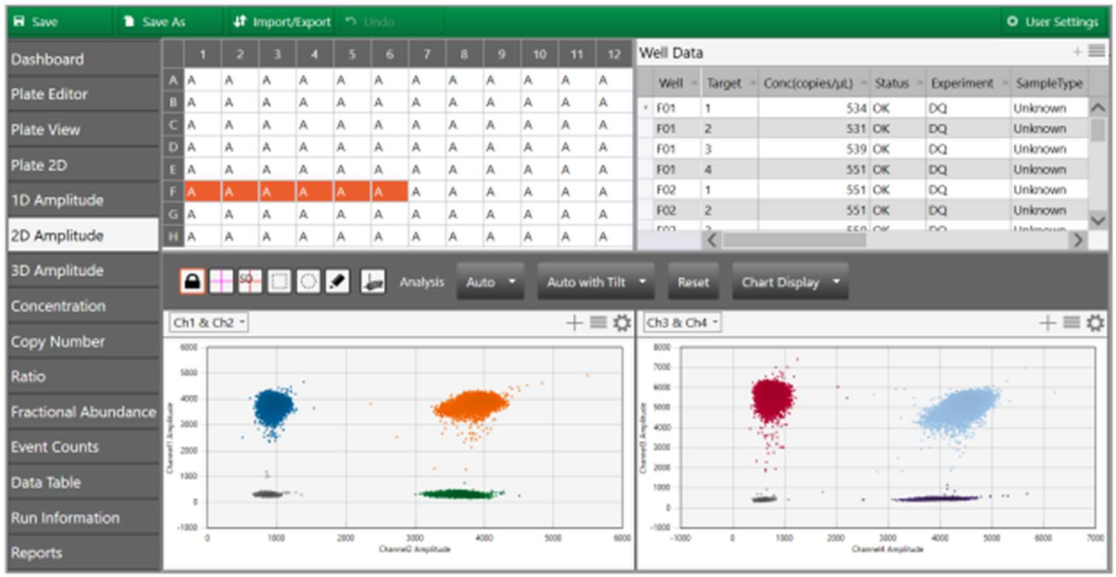
BIO-RAD EX.1009.296

Claims	Exemplary Disclosure of Accused Product
	According to at least the example above, the processor controlled analyzer (e.g. an integrated touch screen computer or standalone computer running the QX ONE software) receives, from the multi-channel detector, a cumulative signal based on the first (e.g., FAM emission wavelength), second (e.g., HEX emission wavelength), third (e.g., Cy5 emission wavelength), and fourth (e.g., Cy5.5 emission wavelength) electromagnetic signals, both literally and under the doctrine of equivalents.
[1.c.2.A] apply a decoding matrix to the cumulative signal to unambiguously detect the presence or absence of at least each of M analytes	The processor controlled analyzer applies a decoding matrix to the cumulative signal to unambiguously detect the presence or absence of at least each of M analytes, both literally and under the doctrine of equivalents. For example, the processor controlled analyzer (e.g. an integrated touch screen computer or standalone computer running the QX ONE software) presents analysis data in different formats, including chromatograms, wherein threshold lines are used to unambiguously distinguish positive or negative droplet counts as indications of the presence or absence of analytes. *See, e.g.*:

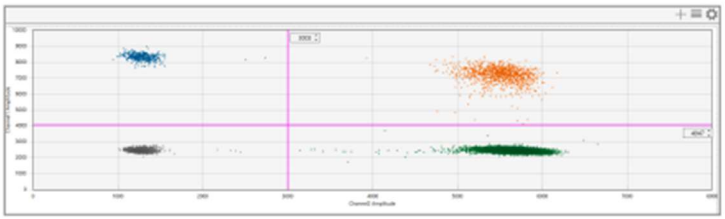
BIO-RAD EX.1009.297

Claims	Exemplary Disclosure of Accused Product
	 Chapter 4 Data Analysis Methodology As summarized in Chapter 3, Data Analysis Module Overview, the software presents analysis data in several different formats. When you open the data file in the Analysis module, you can view the data with the automatic analysis thresholds and calculations or you can choose different options to recalculate, replot, or recolor the results. Data is shown in the following formats: ■ Amplitude, which shows each droplet in each well in a scatter plot, in one-dimensional, two-dimensional, or three-dimensional views ■ Data visualizations, which show estimated target molecule concentration, ratio, and fractional abundance of one target against another target based on selected error model and confidence interval **Note:** Copy number variation is also calculated if the user ran a CNV experiment. ■ Event counts, which are precise counts of positive and negative droplets for each target in each well CT0478876 at CT0478978

BIO-RAD EX.1009.298

Claims	Exemplary Disclosure of Accused Product
	2D Amplitude For the wells you select in the analysis view of the Plate Editor, the 2D Amplitude window illustrates fluorescence for four targets. The images in this section display data from an experiment involving four targets. Each plot shows individual cluster representations for first and second target positive droplets, droplets containing both targets, and droplets containing neither target.  CT0478876 at CT0478990 The processor controlled analyzer (e.g. an integrated touch screen or standalone computer running the QX ONE software) utilizes thresholds. *See, e.g.*:

BIO-RAD EX.1009.299

Claims	Exemplary Disclosure of Accused Product
	Note: Well data must meet certain quality metrics (minimum of 10,000 droplets) before QX ONE Software will automatically calculate a threshold above which droplets are considered positive. You can use the manual thresholding tools to ensure correct quantification of positive and negative droplets. **To use the 2D amplitude tools** 1. Open your data file in the Analysis module and select the 2D Amplitude tab. 2. Choose a threshold line or cluster option. **Note:** Use the cluster option when the data is not orthogonal and the threshold lines do not adequately divide the populations of droplets. ■ To adjust threshold lines, select a threshold line type () and then tap in the plot to apply crosshair lines. Use any of the following methods: □ Drag the lines horizontally or vertically in the plot area. □ Enter a new threshold value in the boxes at the end of each threshold line. □ Tap the up and down arrows to incrementally adjust the numbers.  CT0478876 at CT0478991

BIO-RAD EX.1009.300

Claims	Exemplary Disclosure of Accused Product
	The processor controlled analyzer (e.g. an integrated touch screen computer or standalone computer running the QX ONE software) recognizes certain assay types, including Amplitude Multiplex assay types. *See, e.g.*: Appendix A Experiment Examples This section contains setup and analysis information for the following sample experiments: ■ Direct Quantification, using the Amplitude Multiplex assay type ■ Copy Number Variation, using the Probe Mix Triplex assay type ■ Drop-Off, using the Basic Drop-off Assay type for genome edit detection ■ Direct Quantification, using the Advanced Classification Method assay type CT0478876 at CT0479044 When analyzing Amplitude Multiplex assay types, the processor controlled analyzer (e.g. an integrated touch screen computer or standalone computer running the QX ONE software) uses the thresholds to set the boundaries of discrete signal intensity levels discrete channel intensity levels (e.g., None, Hi, Lo). The processor controlled analyzer assigns up to M=8 targets to the discrete channel intensity levels across the QX ONE's four channels. *See, e.g.*:

BIO-RAD EX.1009.301

Claims	Exemplary Disclosure of Accused Product

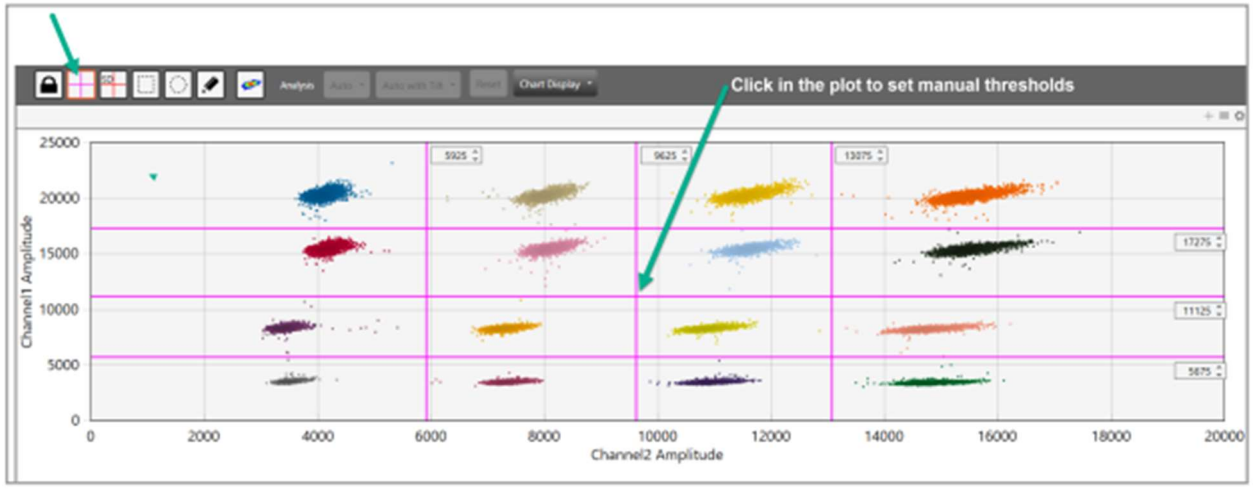
Table 8. Fluorophore options

Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4
Single target per channel, for1 to 4 targets	FAM None	HEX VIC None	Cy5 None	Cy5.5 None
Amplitude multiplex, for 1 to 8 targets	FAM Hi FAM Lo None	HEX Hi HEX Lo VIC Hi VIC Lo None	Cy5 Hi Cy 5 Lo None	Cy5.5 Hi Cy 5.5 Lo None
Probe mix triplex, for 6 targets	FAM None	HEX VIC None	Cy5 None	Cy5.5 None
Advanced classification	FAM +1-10 None	HEX 1 to 10 VIC 1 to 10 None	Cy5 1 to 10 None	Cy5.5 1 to 10 None

CT0478876 at CT0478924

The boundaries of these discrete channel intensity levels are set by thresholds. *See, e.g.*:

BIO-RAD EX.1009.302

Claims	Exemplary Disclosure of Accused Product
	 CT0478876 at CT0479047 The thresholds above thus are understood to discretize the signal intensity values measured on each of the two channels on each plot into four levels 0, 1, 2, and 3:

BIO-RAD EX.1009.303

Claims	Exemplary Disclosure of Accused Product
	 CT0478876 at CT0479047 (notation of discretized channel signal intensity levels added, threshold line emphasis added) Caltech herein incorporates by reference its analysis from Appendix A at [1.c.2.A]. In those elements, Caltech explained how the interface and functionality of the QX Manager Software of the QX600, both literally and under the doctrine of equivalents, applies a decoding matrix to allocate the target combinations among the available regions formed by discretized signal channel levels. On information and belief, at least with respect to Amplitude Multiplex assay type, the QX ONE Software interface and functionality is substantially identical to the interface and functionality of the QX Manager Software of the QX600. *Compare* CT0479068 at CT0479250–CT0479262 *with* CT0478876 at CT0479044– CT0479057. As such, reference to the analysis provided in Appendix A at [1.c.2.A] may be taken to understand how the following coding tables were derived.

BIO-RAD EX.1009.304

<table>
<tr><th>Claims</th><th>Exemplary Disclosure of Accused Product</th></tr>
<tr><td></td><td>Upon naming of the target analytes in the QX ONE Software, the processor controlled analyzer (e.g. an integrated touch screen computer or standalone computer running the QX ONE software) automatically assigns positive droplet readings within specific regions of the chromatogram to specific combinations of the named target analytes.

On information and belief, the processor controlled analyzer (e.g. an integrated touch screen computer or standalone computer running the QX ONE software) applies a decoding matrix to assign potential target analyte combinations to the available regions formed by the discretized signal channel levels, and then droplet data with signal channel levels falling within a given discretized signal channel region is associated with the target analyte combination assigned to that region.

Alternatively, or in addition, the processor controlled analyzer (e.g. an integrated touch screen computer or standalone computer running the QX ONE software) uses a function substantially the same as applying a decoding matrix to the cumulative signal (e.g., assigning the droplet data to target analyte combinations according to how fluorophore intensity levels in the droplet data relate to defined thresholds), in the same way of using cumulative signal data based on the first, second, third, and fourth electromagnetic signals to achieve the same result of unambiguously detecting the presence or absence of at least each of M analytes.

The decoding matrix for channels 1 and 2 may be represented as the following:

<table>
<tr><th>Analyte</th><th>FAM</th><th>HEX</th></tr>
<tr><td>a</td><td>1</td><td>0</td></tr>
<tr><td>b</td><td>2</td><td>0</td></tr>
<tr><td>c</td><td>0</td><td>1</td></tr>
<tr><td>d</td><td>0</td><td>2</td></tr>
</table>
</td></tr>
</table>

BIO-RAD EX.1009.305

<table>
<tr><th>Claims</th><th>Exemplary Disclosure of Accused Product</th></tr>
<tr><td></td><td>Expanding this same analysis to the default target combination assignments to all four channels as provided by the QX ONE Software interface, the following larger decoding table covering eight target analytes (a, b, c, d, e, f, g, and h) may be derived:

<table>
<tr><th>Analyte</th><th>FAM</th><th>HEX</th><th>Cy5</th><th>Cy5.5</th></tr>
<tr><td>a</td><td>1</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>b</td><td>2</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>c</td><td>0</td><td>1</td><td>0</td><td>0</td></tr>
<tr><td>d</td><td>0</td><td>2</td><td>0</td><td>0</td></tr>
<tr><td>e</td><td>0</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>f</td><td>0</td><td>0</td><td>2</td><td>0</td></tr>
<tr><td>g</td><td>0</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>h</td><td>0</td><td>0</td><td>0</td><td>2</td></tr>
</table>

According to at least the examples above, the processor controlled analyzer (e.g. an integrated touch screen computer or standalone computer running the QX ONE software) applies a decoding matrix to the cumulative signal (e.g. assigns target analyte combinations to discrete signal channel levels and associates droplet data with target analyte combinations according to signal channel levels within the droplet data) to unambiguously detect the presence or absence of at least each of M (e.g., M=8) analytes, both literally and under the doctrine of equivalents.</td></tr>
<tr><td>[1.c.2.B]</td><td>The processor controlled analyzer applies the decoding matrix to the cumulative signal data by associating for each analyte, a first value in a first component of the cumulative signal and a second value in a second component of the cumulative signal, wherein each first value is an intensity or range of intensities and each second value is a wavelength or a range of wavelengths, and wherein the</td></tr>
</table>

BIO-RAD EX.1009.306

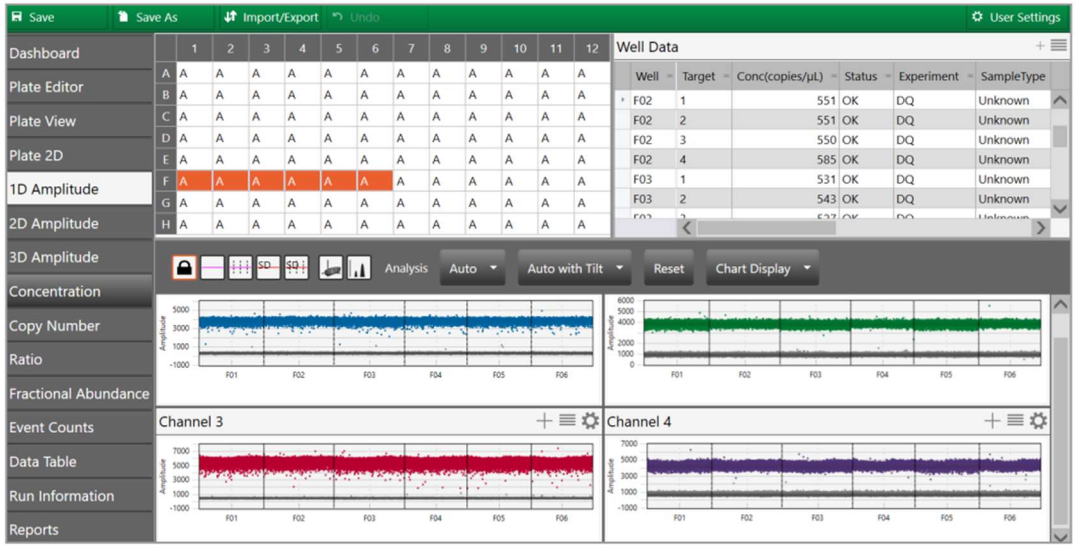
Claims	Exemplary Disclosure of Accused Product
by associating, for each analyte, a first value in a first component of the cumulative signal and a second value in a second component of the cumulative signal, wherein each first value is an intensity or range of intensities and each second value is a wavelength or a range of wavelengths, and wherein the second values comprise the first, second, third, and fourth wavelengths, and	second values comprise the first, second, third, and fourth wavelengths, both literally and under the doctrine of equivalents. For example, as explained in [1.c.2.A] above, the processor controlled analyzer (e.g. an integrated touch screen computer or standalone computer running the QX ONE software) associates signal intensity levels with certain combinations of analytes. The QX ONE streams the droplets single file in front of the multi-channel detector which measures excited fluorophores as positive or negative droplets to quantify target DNA concentration. *See, e.g.*: Chapter 2 QX ONE Droplet Digital PCR System The QX ONE™ Droplet Digital PCR System combines droplet generation, thermal cycling, and droplet reading technologies into a single instrument, as described below: ■ The droplet generator partitions samples into approximately 20,000 uniform droplets. ■ The internal thermal cycler performs repeated heating and cooling processes to amplify one or more copies of a particular DNA segment in each droplet. ■ The droplet reader streams the droplets in single file, and then counts the fluorescent positive and negative droplets to calculate and quantify target DNA concentration. CT0478736 at CT0478758 The multi-channel detector detects electromagnetic signals generated by four different fluorophores (e.g., FAM, HEX, Cy5, and Cy5.5). *See, e.g.*:

BIO-RAD EX.1009.307

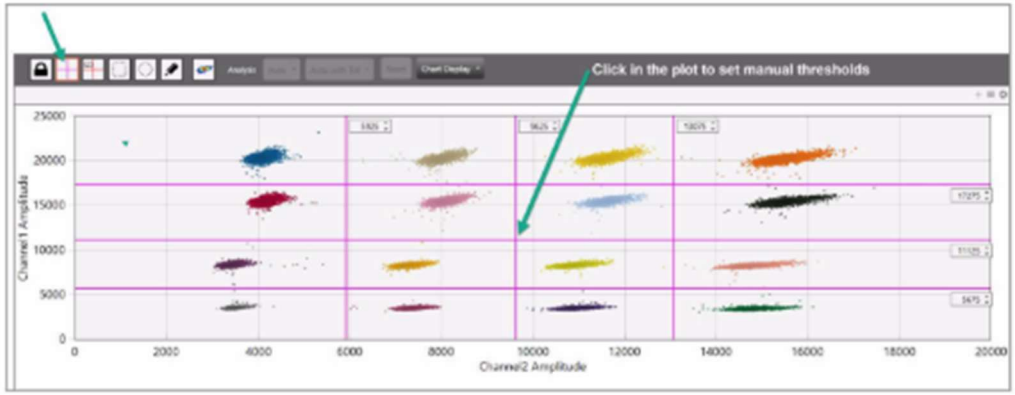
Claims	Exemplary Disclosure of Accused Product
	Instrument Specifications **Table 3. QX ONE Droplet Digital PCR System instrument specifications** CT0478736 at CT0478760

Element	Specifications
Size (W x D x H)	122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor
Weight	100 kg (220 lb) (includes the monitor but not reagents)
Capacity	5 plates
Samples	Up to 96 per plate
Partitions per sample	Approximately 20,000 droplets
Detection channels	4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel
Thermal gradient	Yes
Onboard computer	24 in, 1920 x 1080 resolution Holds data for approximately 100 plates
Time to results	6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol
Benchtop requirements	The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. **Important:** Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis.

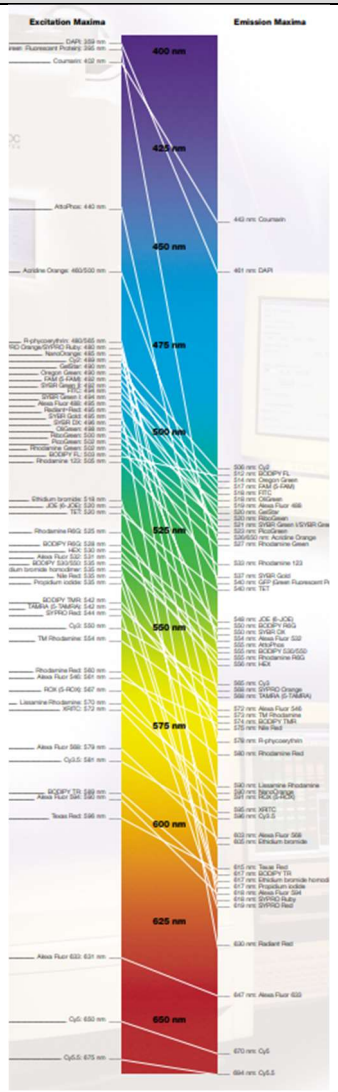
BIO-RAD EX.1009.308

Claims	Exemplary Disclosure of Accused Product
	The QX ONE cumulative signal includes signal intensity (e.g., amplitude) values for each fluorophore wavelength. *See e.g.*: **1D Amplitude** Use the 1D Amplitude analysis window to view droplet amplitudes for each channel, in selection order, for each well selected in the Well Selector.  CT0478876 at CT0478985

BIO-RAD EX.1009.309

Claims	Exemplary Disclosure of Accused Product
	 CT0478876 at CT0479047 Each of the fluorophores has a specific emission wavelength or a range of wavelengths. The emission wavelength for FAM is 517 nm, the emission wavelength for HEX is 556 nm, the emission wavelength for Cy5 is 670 nm, and the emission wavelength for Cy5.5 is 694 nm. *See, e.g.*:

BIO-RAD EX.1009.310

Claims	Exemplary Disclosure of Accused Product
	

Claims	Exemplary Disclosure of Accused Product
	CT0479364 at CT0479364 According to at least the above examples, the processor controlled analyzer (e.g., a touch screen computer or standalone computer running the QX ONE Software) applies the decoding matrix (e.g. assigns target analyte combinations to discrete signal channel levels and associates droplet data with target analyte combinations according to signal channel levels within the droplet data) to the cumulative signal data by associating for each analyte, a first value (e.g., a FAM fluorescence intensity value), in a first component of the cumulative signal and a second value (e.g., a FAM emission wavelength value), in a second component of the cumulative signal, wherein each first value (e.g. a FAM fluorescence intensity value, a HEX fluorescence intensity value, a Cy5 fluorescence intensity value, a Cy5.5 fluorescence intensity value), is an intensity or range of intensities and each second value (e.g., a FAM emission wavelength value, a HEX emission wavelength value, a Cy5 emission wavelength value, a Cy5.5 emission wavelength value) is a wavelength or a range of wavelengths, and wherein the second values comprise the first (e.g., FAM wavelength, or 517 nm), second (e.g., HEX wavelength, or 556 nm), third (e.g., Cy5 wavelength, or 670 nm) and fourth (e.g., Cy5.5 wavelength, or 694 nm) wavelengths, both literally and under the doctrine of equivalents.
[1.c.2.C] the determination is made without immobilization, mass spectrometry or melting curve analysis;	Caltech incorporates the discussion from elements of claim 1, *supra,* in their entirety. As discussed in relation to those elements, the unambiguous determination of the presence or absence of at least each of the M analytes in the sample is made without immobilization, mass spectrometry, or melting curve analysis.
[1.c.2.D]	The processor controlled analyzer makes determinations wherein, for the positive integer M, M=C*log2(F+1), both literally and under the doctrine of equivalents.

BIO-RAD EX.1009.312

Claims	Exemplary Disclosure of Accused Product
wherein for the positive integer M, M=C*log2(F+1),	For example, where intensity data is collected from all four channels, the number of second values, C (e.g., fluorophore wavelengths) is four. *See, e.g.*:

Instrument Specifications

Table 3. QX ONE Droplet Digital PCR System instrument specifications

Element	Specifications
Size (W x D x H)	122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor
Weight	100 kg (220 lb) (includes the monitor but not reagents)
Capacity	5 plates
Samples	Up to 96 per plate
Partitions per sample	Approximately 20,000 droplets
Detection channels	4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel
Thermal gradient	Yes
Onboard computer	24 in, 1920 x 1080 resolution Holds data for approximately 100 plates
Time to results	6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol
Benchtop requirements	The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. **Important:** Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis.

BIO-RAD EX.1009.313

Claims	Exemplary Disclosure of Accused Product
	CT0478736 at CT0478760 The processor controlled analyzer (e.g., a touch screen or standalone computer running the QX ONE Software) applies a decoding matrix to the cumulative signal (e.g. assigns target analyte combinations to discrete signal channel levels and associates droplet data with target analyte combinations according to signal channel levels within the droplet data), such that maximum cumulative intensity for any channel, F, is three. *See, e.g.*:  CT0478876 at CT0479047, at 172 (notation of discretized channel signal intensity levels added, threshold line emphasis added) Plugging C=4 and F=3 into the equation M=C*log2(F+1), calculates an analyte count of eight: M=C*log2(F+1)

BIO-RAD EX.1009.314

Claims	Exemplary Disclosure of Accused Product
	M=4*log2(3+1) M=4*log2(4) M=4*2 M=8 This is consistent with the number of analytes unambiguously determined to be present or absent when the QX ONE uses four channels, which is eight analytes. *See e.g.*:

BIO-RAD EX.1009.315

Claims	Exemplary Disclosure of Accused Product
	Assay Types and Fluorophores QX ONE Software offers the assay methods explained in Table 10. **Table 10. Assay types** **Assay type** / **Description** Single target per channel: Method assuming up to four probe colors (FAM, HEX/VIC, Cy5 and Cy5.5) and up to four targets per well, with a single target detected per channel. This is the default assay type, with the maximum four rows displayed. You can delete up to three rows. Amplitude multiplex: Method to increase multiplexing up to eight targets per well, with one or two targets detected per channel. The maximum of eight rows are displayed. You can delete up to seven rows. Probe mix triplex: Triplex mode that allows two groups of three targets each. Group 1 has one target in FAM, one target in HEX/VIC, and one target in both channels. Group 2 has one target in Cy5, one target in Cy5.5, and one target in both. You can delete a group, but not the individual targets within the group. **Note:** The channels within a group are not interchangeable; therefore FAM is always paired with HEX/VIC and so forth. Advanced classification: Method to increase multiplexing rate in any combination of targets detected per channel. This method requires manual classification of droplets. Basic drop-off: This mode allows two groups of two targets each. The first group appears by default, with one target in FAM and HEX/VIC and one target in FAM or HEX/VIC. This assay type is available only for the DOF experiment type. You can add one more group of two targets, with one target in Cy5 and Cy5.5, one target in Cy5 or Cy5.5. You can delete a group, but not the individual targets in the group. **Note:** The channels within a group are not interchangeable; therefore FAM is always paired with HEX/VIC and so forth. CT0478736 at CT0478804

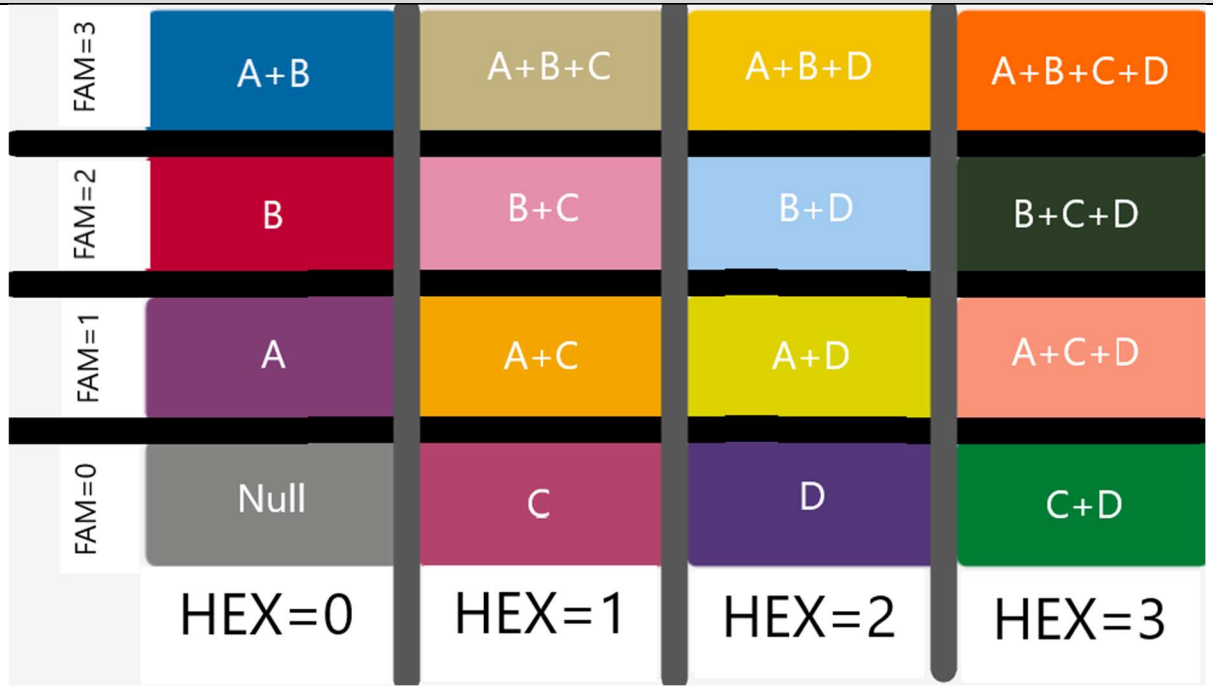
BIO-RAD EX.1009.316

Claims	Exemplary Disclosure of Accused Product
	According at least to the above examples, the processor controlled analyzer (e.g., a touch screen computer or standalone computer running the QX ONE Software) makes determinations wherein, for the positive integer M (e.g., 8), M=C*log2(F+1) (e.g., 8=4*log2(3+1)), both literally and under the doctrine of equivalents.
[1.c.2.E] F is a positive integer and is equal to the maximum cumulative intensity of the first component of the signal, for any second value, when all of the analytes are present, and	The processor controlled analyzer makes determinations wherein F is a positive integer and is equal to the maximum cumulative intensity of the first component of the signal, for any second value, when all of the analytes are present, both literally and under the doctrine of equivalents. For example, the processor controlled analyzer (e.g., a touch screen computer or standalone computer running the QX ONE Software) applies a decoding matrix to the cumulative signal (e.g. assigns target analyte combinations to discrete signal channel levels and associates droplet data with target analyte combinations according to signal channel levels within the droplet data), such that maximum cumulative intensity for any channel, F, is three. *See, e.g.*:

BIO-RAD EX.1009.317

Claims	Exemplary Disclosure of Accused Product
	 CT0478876 at CT0479047, at 172 (notation of discretized channel signal intensity levels added, threshold line emphasis added) F=3 and three is a positive integer. Where signal intensity on both the FAM and HEX channels exceeds the threshold for a discrete valuation of three, the detection is classified in the upper right orange region, indicating the presence of all analytes (A + B + C + D). *See, e.g.*:

BIO-RAD EX.1009.318

Claims	Exemplary Disclosure of Accused Product
	 CT0478876 at CT0479048, at 173 (white text indicating each unique combination of positive readings for the four analytes (A, B, C, and D) added, black and grey emphasis on threshold lines added, HEX/FAM integer values added) The orange cluster represents droplets containing all of the analytes of the chromatogram. If signal intensity on all channels (e.g., FAM, HEX, Cy5, Cy5.5) exceeds the threshold for a discrete valuation of three, then the sample would be classified as containing all analytes.

BIO-RAD EX.1009.319

Claims	Exemplary Disclosure of Accused Product
	According at least to the above examples, the processor controlled analyzer (e.g., a touch screen computer or standalone computer running the QX ONE Software) makes determinations wherein F is a positive integer (e.g., 3) and is equal to the maximum cumulative intensity of the first component of the signal, for any second value (e.g., intensity at FAM, HEX, Cy5, or Cy5.5 emission wavelengths) when all of the analytes are present, both literally and under the doctrine of equivalents.
[1.c.2.F] C=4, 5, or 6; and	The processor controlled analyzer makes determinations wherein C=4, 5, or 6, both literally and under the doctrine of equivalents. For example, the QX ONE collects intensity data from up to four channels (e.g., FAM, HEX, Cy5, and Cy5.5), so the number of second values, C (e.g., fluorophore wavelengths) may be 1, 2, 3, or 4. *See, e.g.*:

BIO-RAD EX.1009.320

Claims	Exemplary Disclosure of Accused Product
	## Instrument Specifications **Table 3. QX ONE Droplet Digital PCR System instrument specifications** | Element	Specifications
Size (W x D x H)	122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor
Weight	100 kg (220 lb) (includes the monitor but not reagents)
Capacity	5 plates
Samples	Up to 96 per plate
Partitions per sample	Approximately 20,000 droplets
Detection channels	4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel
Thermal gradient	Yes
Onboard computer	24 in, 1920 x 1080 resolution Holds data for approximately 100 plates
Time to results	6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol
Benchtop requirements	The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. **Important:** Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis. | CT0478736 at CT0478760

BIO-RAD EX.1009.321

Claims	Exemplary Disclosure of Accused Product
	According at least to the above examples, the processor controlled analyzer (e.g., a touch screen or standalone computer running the QX ONE Software) makes determinations wherein C=4, 5, or 6 (e.g., 4), both literally and under the doctrine of equivalents.
[1.c.2.G] wherein F+1 is a positive integer and wherein F+1 is a power of 2,	The processor controlled analyzer makes determinations wherein F+1 is a positive integer and wherein F+1 is a power of 2, both literally and under the doctrine of equivalents. For example, the processor controlled analyzer applies a decoding matrix to the cumulative signal (e.g. assigns target analyte combinations to discrete signal channel levels and associates droplet data with target analyte combinations according to signal channel levels within the droplet data), such that maximum cumulative intensity for any channel, F, is three. *See, e.g.*: 

BIO-RAD EX.1009.322

Claims	Exemplary Disclosure of Accused Product
	CT0478876 at CT0479047, at 172 (notation of discretized channel signal intensity levels added, threshold line emphasis added) F=3 in the above examples, and F+1=4. Four is a positive integer and a power of two, e.g. 2^2=4. According at least to the above examples, the processor controlled analyzer (e.g., a touch screen computer or standalone computer running the QX ONE Software) makes determinations wherein F+1 is a positive integer (e.g., 4) and wherein F+1 is a power of 2 (e.g., 4=2^2), both literally and under the doctrine of equivalents.
[1.c.2.H] wherein M is greater than the number of the second values used to encode the analytes (C), the multi-channel detector comprises C channels, and M and C are positive integers.	The processor controlled analyzer makes determinations wherein M is greater than the number of the second values used to encode the analytes (C), the multi-channel detector comprises C channels, and M and C are positive integers, both literally and under the doctrine of equivalents. For example, the QX ONE detects analytes using up to four color channels (e.g., FAM, HEX, Cy5, and Cy5.5) to unambiguously detect up to eight targets. *See, e.g.*:

BIO-RAD EX.1009.323

Instrument Specifications

Table 3. QX ONE Droplet Digital PCR System instrument specifications

Element	Specifications
Size (W x D x H)	122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor
Weight	100 kg (220 lb) (includes the monitor but not reagents)
Capacity	5 plates
Samples	Up to 96 per plate
Partitions per sample	Approximately 20,000 droplets
Detection channels	4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel
Thermal gradient	Yes
Onboard computer	24 in, 1920 x 1080 resolution Holds data for approximately 100 plates
Time to results	6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol
Benchtop requirements	The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. **Important:** Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis.

CT0478736 at CT0478760

BIO-RAD EX.1009.324

Claims	Exemplary Disclosure of Accused Product
	Table 8. Fluorophore options CT0478876 at CT0478924

Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4
Single target per channel, for1 to 4 targets	FAM None	HEX VIC None	Cy5 None	Cy5.5 None
Amplitude multiplex, for 1 to 8 targets	FAM Hi FAM Lo None	HEX Hi HEX Lo VIC Hi VIC Lo None	Cy5 Hi Cy 5 Lo None	Cy5.5 Hi Cy 5.5 Lo None
Probe mix triplex, for 6 targets	FAM None	HEX VIC None	Cy5 None	Cy5.5 None
Advanced classification	FAM +1-10 None	HEX 1 to 10 VIC 1 to 10 None	Cy5 1 to 10 None	Cy5.5 1 to 10 None

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Claims	Exemplary Disclosure of Accused Product
	Where intensity data is collected from four colors, the number of second values, C (e.g., fluorophore wavelengths), is four. The number of analytes (e.g., targets) unambiguously determined, M, is up to eight when the number of second values used to encode the analytes, C, is four. Eight is greater than four, and eight and four are both positive integers. According at least to the above examples, the processor controlled analyzer (e.g., a touch screen or standalone computer running the QX ONE Software) makes determinations wherein M (e.g., eight or six) is greater than the number of the second values used to encode the analytes (C) (e.g., four), and M and C are positive integers, both literally and under the doctrine of equivalents.
[5.pre] 5. The system of claim 1, wherein	Caltech incorporates the discussion from claim 1, *supra,* in its entirety. As discussed therein, the QX ONE is a system comprising each and every element of claim 1, both literally and under the doctrine of equivalents.
[5.a] F=3.	The processor controlled analyzer makes determinations wherein F= 3, both literally and under the doctrine of equivalents. For example, the processor controlled analyzer (e.g., a touch screen or standalone computer running the QX ONE Software) applies a decoding matrix to the cumulative signal (e.g. assigns target analyte combinations to discrete signal channel levels and associates droplet data with target analyte combinations according to signal channel levels within the droplet data), such that maximum cumulative intensity for any channel, F, is three. *See, e.g.*:

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Claims	Exemplary Disclosure of Accused Product
	 CT0478876 at CT0479047 (notation of discretized channel signal intensity levels added, threshold line emphasis added) According at least to the above examples, the processor controlled analyzer processor controlled analyzer (e.g., a touch screen or standalone computer running the QX ONE Software) makes determinations wherein F=3, both literally and under the doctrine of equivalents.
[6.pre] 6. The system of claim 5, wherein	Caltech incorporates the discussion from claim 5, *supra,* in its entirety. As discussed therein, the QX ONE is a system comprising each and every element of claim 5, both literally and under the doctrine of equivalents.

BIO-RAD EX.1009.327

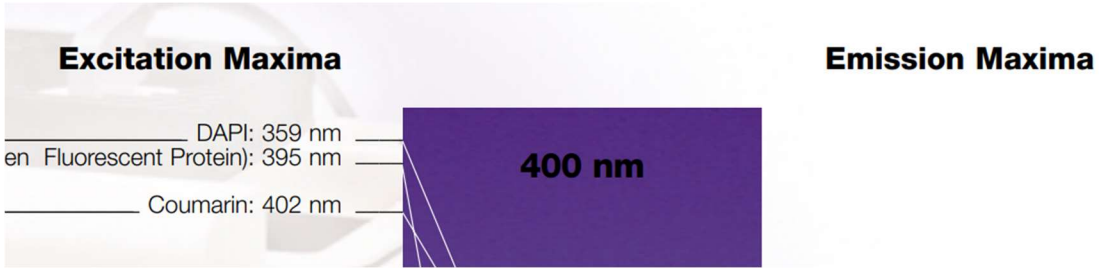
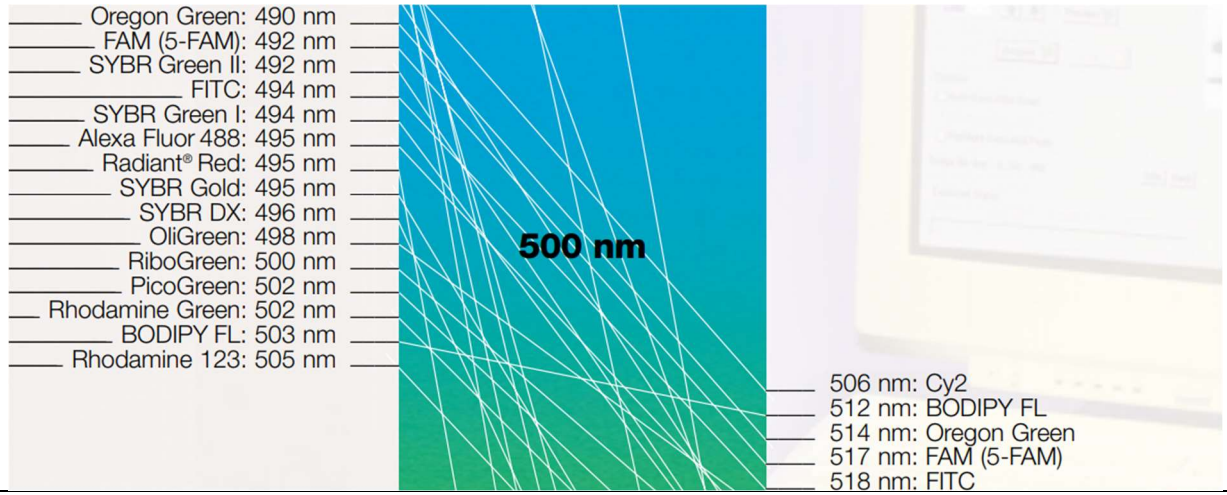
Claims	Exemplary Disclosure of Accused Product
[6.a] the multiple fluorophores is selected from the group consisting of a blue fluorophore, a green fluorophore, a yellow fluorophore, a red fluorophore, and any combinations thereof.	The QX ONE detects electromagnetic waves at the emission wavelength of multiple fluorophores, wherein the multiple fluorophores is selected from the group consisting of a blue fluorophore, a green fluorophore, a yellow fluorophore, a red fluorophore, and any combinations thereof, both literally and under the doctrine of equivalents. For example, the QX ONE detects electromagnetic waves at the emission wavelength of multiple fluorophores (e.g., FAM, HEX, Cy5, and Cy5.5). *See, e.g.*:

BIO-RAD EX.1009.328

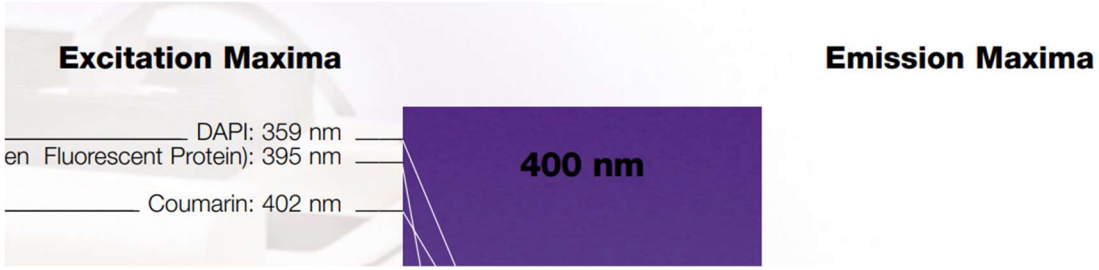
Claims	Exemplary Disclosure of Accused Product
	Instrument Specifications **Table 3. QX ONE Droplet Digital PCR System instrument specifications** CT0478736 at CT0478760

Element	Specifications
Size (W x D x H)	122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor
Weight	100 kg (220 lb) (includes the monitor but not reagents)
Capacity	5 plates
Samples	Up to 96 per plate
Partitions per sample	Approximately 20,000 droplets
Detection channels	4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel
Thermal gradient	Yes
Onboard computer	24 in, 1920 x 1080 resolution Holds data for approximately 100 plates
Time to results	6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol
Benchtop requirements	The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. **Important:** Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis.

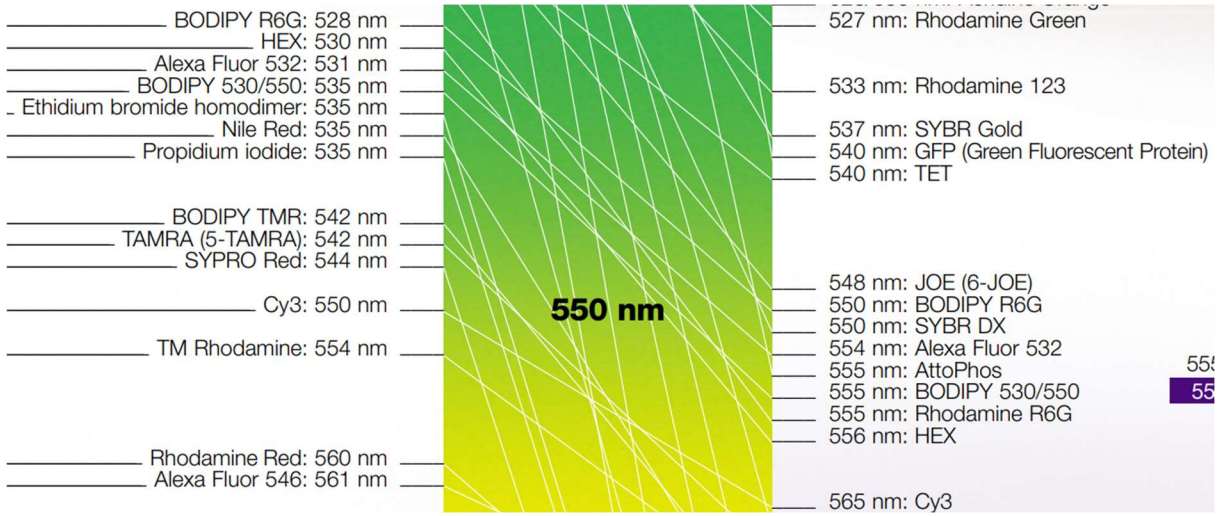
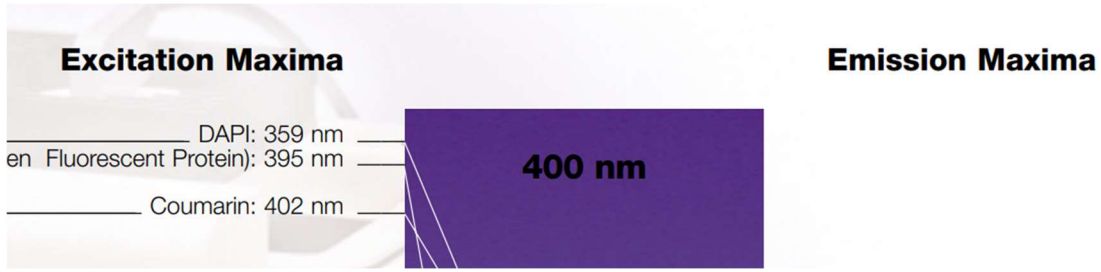
BIO-RAD EX.1009.329

Claims	Exemplary Disclosure of Accused Product
	The FAM fluorophore is a green fluorophore. *See, e.g.*:  CT0479364 at CT0479364 

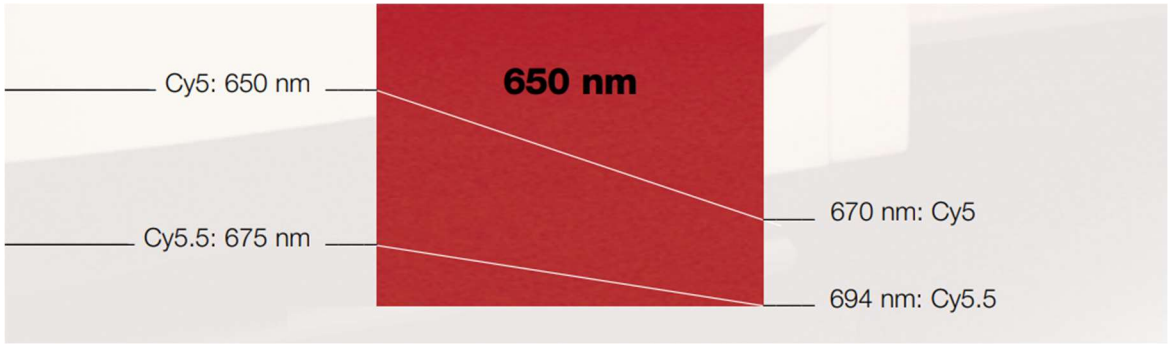
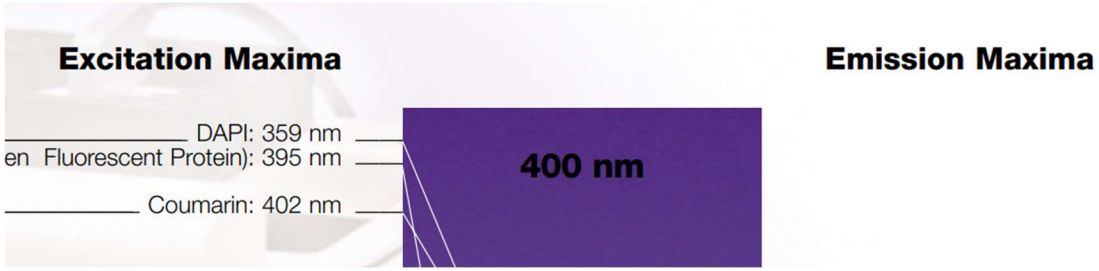
BIO-RAD EX.1009.330

Claims	Exemplary Disclosure of Accused Product
	CT0479364 at CT0479364 The HEX fluorophore is a green and yellow fluorophore. *See, e.g.*:  CT0479364 at CT0479364

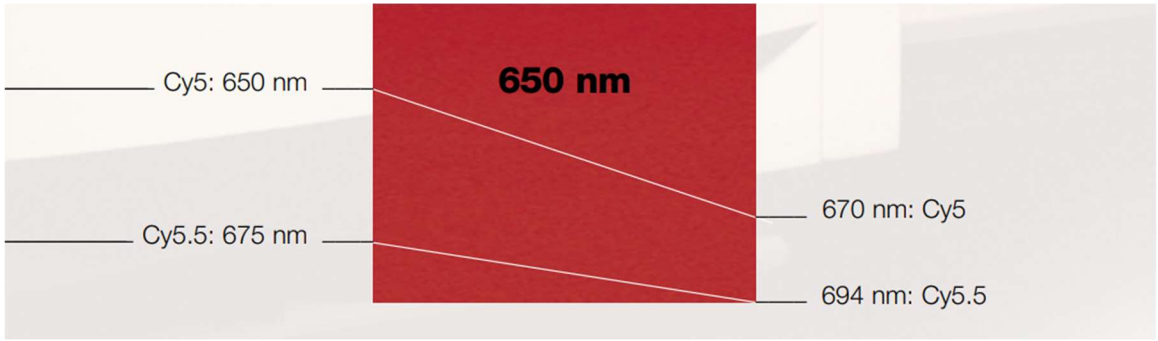
BIO-RAD EX.1009.331

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364 The Cy5 fluorophore is a red fluorophore. *See, e.g.*:  CT0479364 at CT0479364

BIO-RAD EX.1009.332

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364 The Cy5.5 fluorophore is a red fluorophore. *See, e.g.*:  CT0479364 at CT0479364

BIO-RAD EX.1009.333

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364 According to at least the above examples, the QX ONE detects electromagnetic waves at the emission wavelength of multiple fluorophores (e.g., FAM, HEX, Cy5, and Cy5.5), wherein the multiple fluorophores is selected from the group consisting of a blue fluorophore, a green fluorophore (e.g., FAM), a yellow fluorophore, a red fluorophore (e.g., Cy5, Cy5.5), and any combinations thereof (e.g., HEX which is a green and yellow fluorophore), both literally and under the doctrine of equivalents.
[7.pre] 7. The system of claim 5, wherein	Caltech incorporates the discussion from claim 5, *supra,* in its entirety. As discussed therein, the QX ONE is a system comprising each and every element of claim 5, both literally and under the doctrine of equivalents.

BIO-RAD EX.1009.334

Claims	Exemplary Disclosure of Accused Product
[7.a] the multiple fluorophores is selected from the group consisting of a fluorescein amidite (FAM), a cyanine 3 (Cy3), a carboxy-X-rhodamine (ROX), a cyanine 5 (Cy5), a cyanine 5.5 (Cy5.5), and any combinations thereof.	The QX ONE detects electromagnetic waves at the emission wavelength of multiple fluorophores, wherein the multiple fluorophores is selected from the group consisting of a fluorescein amidite (FAM), a cyanine 3 (Cy3), a carboxy-X-rhodamine (ROX), a cyanine 5 (Cy5), a cyanine 5.5 (Cy5.5), and any combinations thereof, both literally and under the doctrine of equivalents. For example, the QX ONE detects electromagnetic waves at the emission wavelength of multiple fluorophores (e.g., FAM, Cy5, and Cy5.5). *See, e.g.*:

BIO-RAD EX.1009.335

Claims	Exemplary Disclosure of Accused Product
	Instrument Specifications **Table 3. QX ONE Droplet Digital PCR System instrument specifications** **Element** / **Specifications** Size (W x D x H) — 122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor Weight — 100 kg (220 lb) (includes the monitor but not reagents) Capacity — 5 plates Samples — Up to 96 per plate Partitions per sample — Approximately 20,000 droplets Detection channels — 4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel Thermal gradient — Yes Onboard computer — 24 in, 1920 x 1080 resolution Holds data for approximately 100 plates Time to results — 6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol Benchtop requirements — The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. Important: Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis. CT0478736 at CT0478760

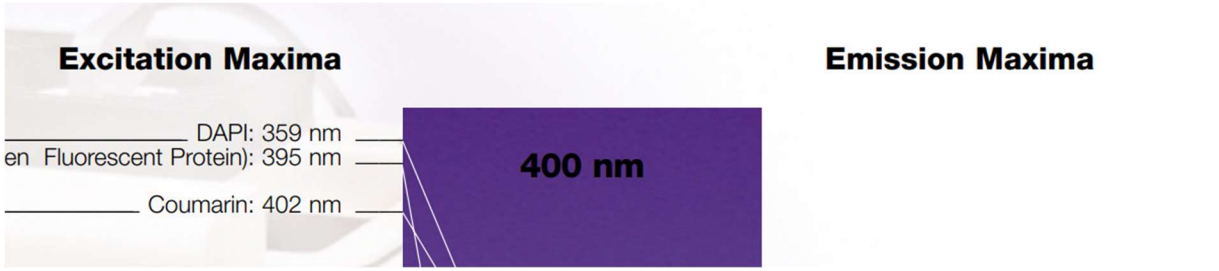
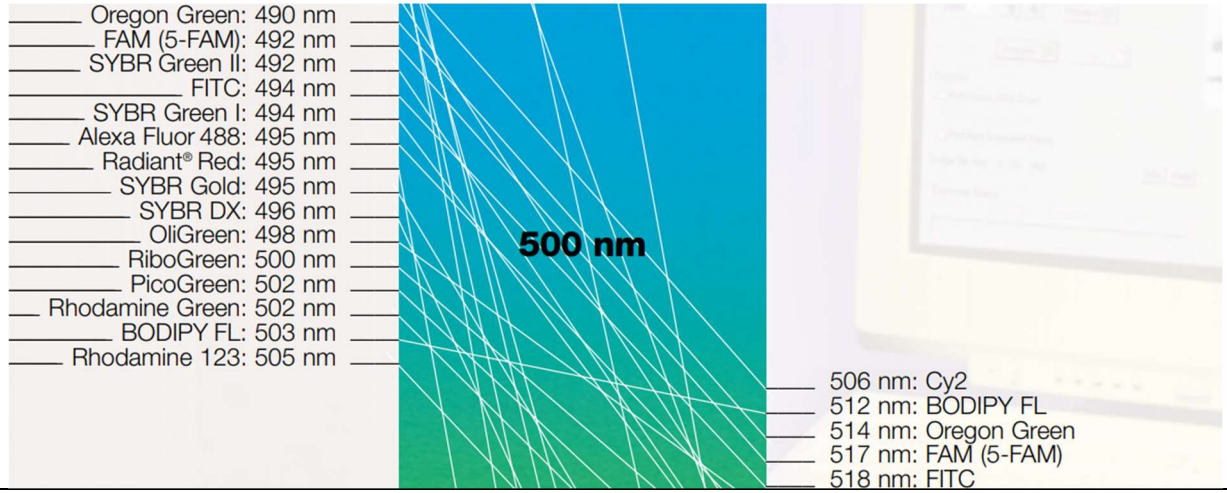
BIO-RAD EX.1009.336

Claims	Exemplary Disclosure of Accused Product
	According to at least the above examples, the QX ONE detects electromagnetic waves at the emission wavelength of multiple fluorophores (e.g., FAM, Cy5, and Cy5.5), wherein the multiple fluorophores is selected from the group consisting of a fluorescein amidite (FAM), a cyanine 3 (Cy3), a carboxy-X-rhodamine (ROX), a cyanine 5 (Cy5), a cyanine 5.5 (Cy5.5), and any combinations thereof, both literally and under the doctrine of equivalents.
[8.pre] 8. The system of claim 5, wherein	Caltech incorporates the discussion from claim 5, *supra,* in its entirety. As discussed therein, the QX ONE is a system comprising each and every element of claim 5, both literally and under the doctrine of equivalents.
[8.a] the multiple fluorophores has a maximum excitation wavelength selected from the group consisting of about 494 nm, about 550 nm, about 567 nm, about 650 nm, about 675 nm, and any combinations thereof.	The QX ONE detects electromagnetic waves at the emission wavelength of multiple fluorophores, wherein the multiple fluorophores has a maximum excitation wavelength selected from the group consisting of about 494 nm, about 550 nm, about 567 nm, about 650 nm, about 675 nm, and any combinations thereof, both literally and under the doctrine of equivalents. For example, the QX ONE detects electromagnetic waves at the emission wavelength of multiple fluorophores (e.g., FAM, Cy5, and Cy5.5). *See, e.g.*:

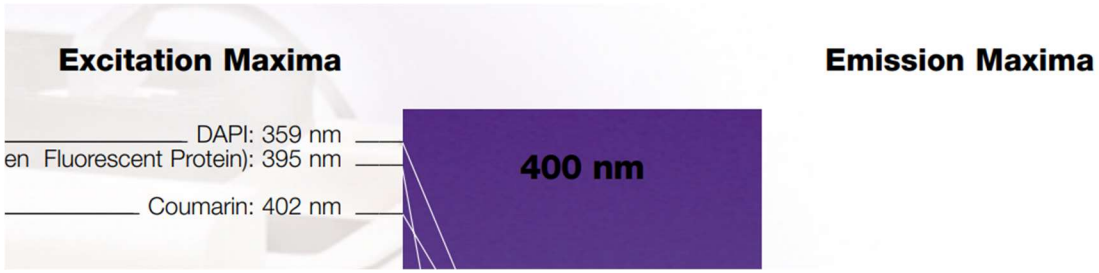
BIO-RAD EX.1009.337

Claims	Exemplary Disclosure of Accused Product
	Instrument Specifications **Table 3. QX ONE Droplet Digital PCR System instrument specifications** **Element** / **Specifications** Size (W x D x H) — 122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor Weight — 100 kg (220 lb) (includes the monitor but not reagents) Capacity — 5 plates Samples — Up to 96 per plate Partitions per sample — Approximately 20,000 droplets Detection channels — 4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel Thermal gradient — Yes Onboard computer — 24 in, 1920 x 1080 resolution Holds data for approximately 100 plates Time to results — 6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol Benchtop requirements — The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. Important: Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis. CT0478736 at CT0478760

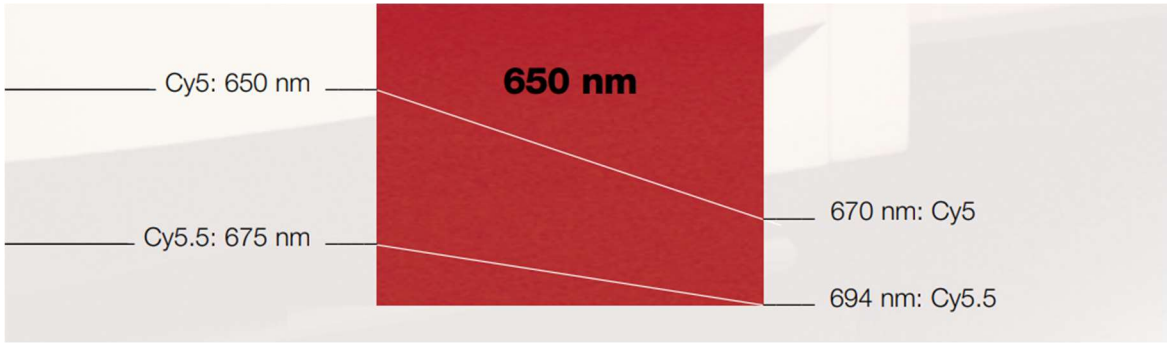
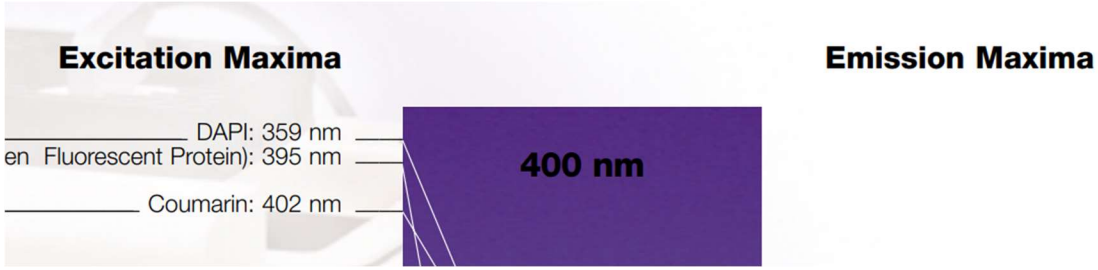
BIO-RAD EX.1009.338

Claims	Exemplary Disclosure of Accused Product
	The FAM fluorophore has a maximum excitation wavelength of about 494 nm (e.g., 492 nm). *See, e.g.*:  CT0479364 at CT0479364 

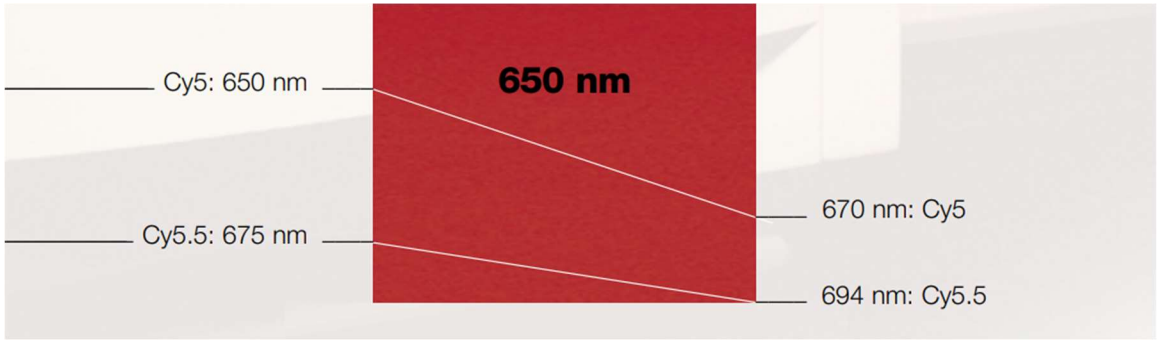
BIO-RAD EX.1009.339

Claims	Exemplary Disclosure of Accused Product
	CT0479364 at CT0479364 The Cy5 fluorophore has a maximum excitation wavelength of about 650 nm (e.g., 650 nm). *See, e.g.*:  CT0479364 at CT0479364

BIO-RAD EX.1009.340

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364 The Cy5.5 fluorophore has a maximum excitation wavelength of about 675 nm (e.g., 675 nm). *See, e.g.*:  CT0479364 at CT0479364

BIO-RAD EX.1009.341

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364 According to at least the above examples, the QX ONE detects electromagnetic waves at the emission wavelength of multiple fluorophores (e.g., FAM, Cy5, and Cy5.5), wherein the multiple fluorophores has a maximum excitation wavelength selected from the group consisting of about 494 nm (e.g., FAM: 492 nm), about 550 nm, about 567 nm, about 650 nm (e.g., Cy5: 650 nm), about 675 nm (e.g., Cy5.5: 675 nm), and any combinations thereof, both literally and under the doctrine of equivalents.
[9.pre] 9. The system of claim 5, wherein	Caltech incorporates the discussion from claim 5, *supra,* in its entirety. As discussed therein, the QX ONE is a system comprising each and every element of claim 5, both literally and under the doctrine of equivalents.

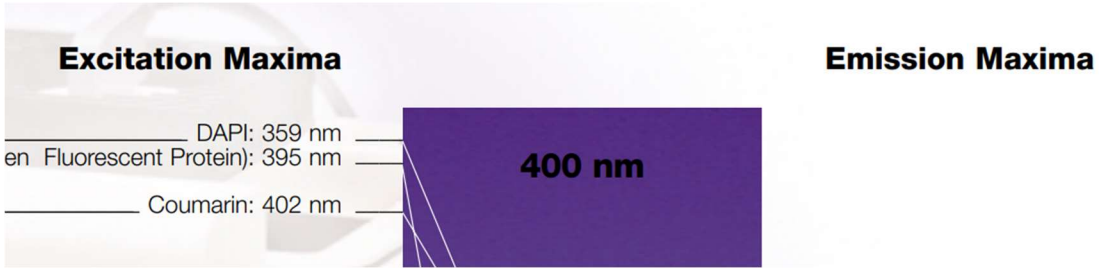
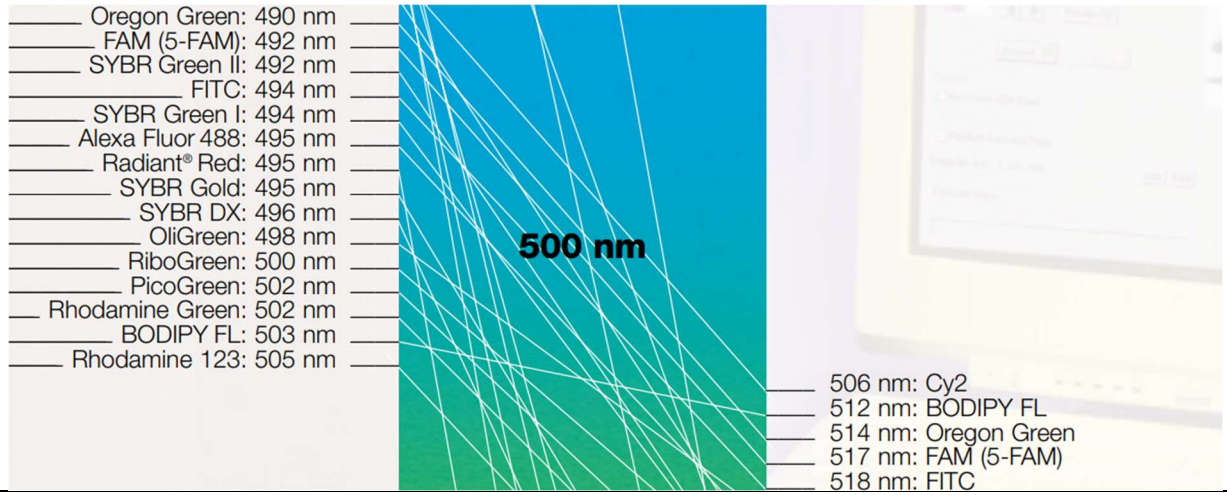
BIO-RAD EX.1009.342

Claims	Exemplary Disclosure of Accused Product
[9.a] the multiple fluorophores has a maximum emission wavelength selected from the group consisting of about 518 nm, about 565 nm, about 591 nm, about 670 nm, about 697 nm, and any combinations thereof.	The QX ONE detects electromagnetic waves at the emission wavelength of multiple fluorophores, wherein the multiple fluorophores has a maximum emission wavelength selected from the group consisting of about 518 nm, about 565 nm, about 591 nm, about 670 nm, about 697 nm, and any combinations thereof, both literally and under the doctrine of equivalents. For example, the QX ONE detects electromagnetic waves at the emission wavelength of multiple fluorophores (e.g., FAM, Cy5, and Cy5.5). *See, e.g.*:

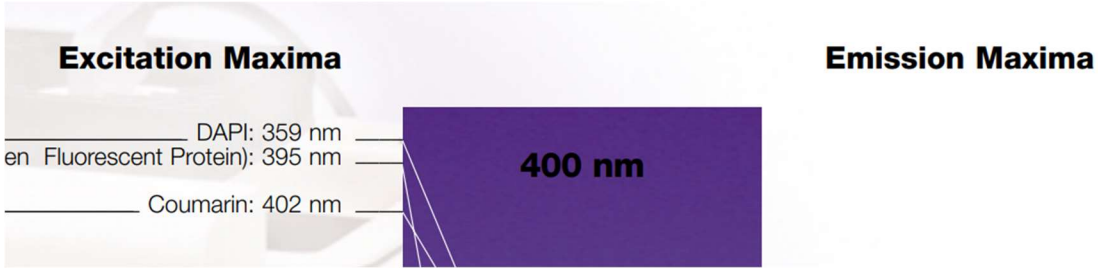
BIO-RAD EX.1009.343

Claims	Exemplary Disclosure of Accused Product
	Instrument Specifications **Table 3. QX ONE Droplet Digital PCR System instrument specifications** **Element** / **Specifications** Size (W x D x H) — 122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor Weight — 100 kg (220 lb) (includes the monitor but not reagents) Capacity — 5 plates Samples — Up to 96 per plate Partitions per sample — Approximately 20,000 droplets Detection channels — 4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel Thermal gradient — Yes Onboard computer — 24 in, 1920 x 1080 resolution Holds data for approximately 100 plates Time to results — 6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol Benchtop requirements — The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. Important: Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis. CT0478736 at CT0478760

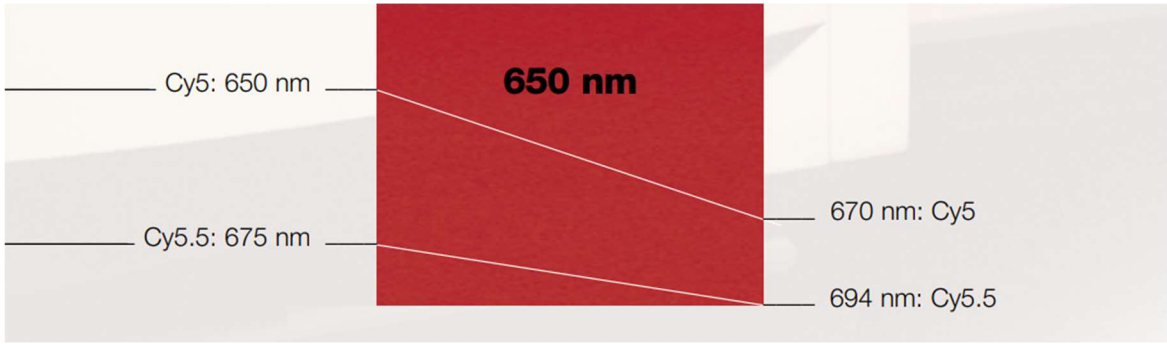
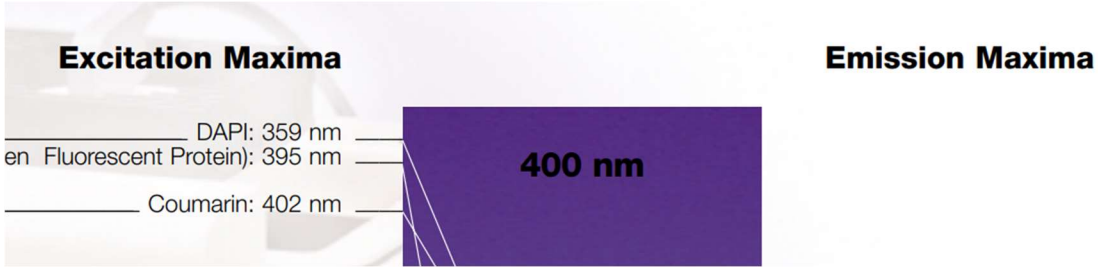
BIO-RAD EX.1009.344

Claims	Exemplary Disclosure of Accused Product
	The FAM fluorophore has a maximum emission wavelength of about 518 nm (e.g., 517 nm). *See, e.g.*:  CT0479364 at CT0479364 

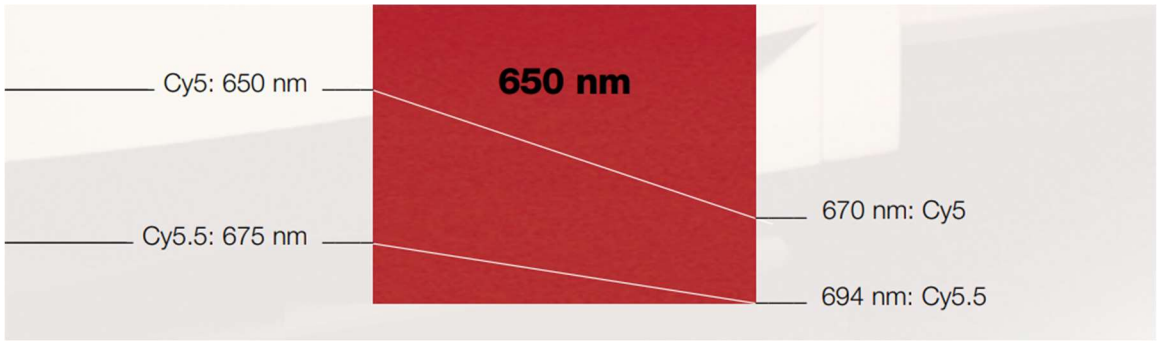
BIO-RAD EX.1009.345

Claims	Exemplary Disclosure of Accused Product
	CT0479364 at CT0479364 The Cy5 fluorophore has a maximum emission wavelength of about 670 nm (e.g., 670 nm). *See, e.g.*:  CT0479364 at CT0479364

BIO-RAD EX.1009.346

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364 The Cy5.5 fluorophore has a maximum emission wavelength of about 697 nm (e.g., 694 nm). *See, e.g.*:  CT0479364 at CT0479364

BIO-RAD EX.1009.347

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364 According to at least the above examples, the QX ONE detects electromagnetic waves at the emission wavelength of multiple fluorophores (e.g., FAM, Cy5, and Cy5.5), wherein the multiple fluorophores has a maximum emission wavelength selected from the group consisting of about 518 nm (e.g., FAM: 517 nm), about 565 nm, about 591 nm, about 670 nm (e.g., Cy5: 670 nm), about 697 nm (e.g., Cy5.5: 694 nm), and any combinations thereof, both literally and under the doctrine of equivalents.
[10.pre] 10. The system of claim 5, wherein	Caltech incorporates the discussion from claim 5, *supra,* in its entirety. As discussed therein, the QX ONE is a system comprising each and every element of claim 5, both literally and under the doctrine of equivalents.

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Claims	Exemplary Disclosure of Accused Product
[10.a] the analyte-specific hybridization probes are labeled with a fluorophore of the multiple fluorophores.	The analyte-specific hybridization probes within the analyte-specific reagent mixture are labeled with a fluorophore of the multiple fluorophores, both literally and under the doctrine of equivalents. For example, the QX ONE ddPCR workflow includes combining a sample (e.g., DNA or RNA) with an analyte-specific reagent mixture (e.g., primers, probes, and Bio-Rad ddPCR supermix). *See, e.g.*: **ddPCR Workflow** The ddPCR process adheres to the following workflow: ■ You prepare your samples for PCR by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermix. ■ A droplet generator fractionates a sample into ~20,000 nanoliter-sized droplets with target and background DNA distributed randomly into the droplets during the partitioning process. ■ Following droplet generation, the droplets are run through a thermal cycler, which performs PCR amplification of the nucleic acid target in each individual droplet. ■ A droplet reader reads each droplet to determine the fraction of positive droplets in the original sample and uses Poisson statistical formulas to determine the absolute quantity. **Note:** Positive droplets containing at least one copy of the target DNA molecule exhibit increased fluorescence compared to negative droplets. CT0478736 at CT0478755

BIO-RAD EX.1009.349

Claims	Exemplary Disclosure of Accused Product
	The analyte-specific reagent mixtures (e.g. primers, probes, and Bio-Rad ddPCR supermix) include certain PCR supermixes for probe-based assays, including hydrolysis probe-based assays. *See, e.g.*:

Supermixes

QX ONE Software offers five PCR supermixes. Each supermix is optimized to deliver maximum PCR efficiency and sensitivity for the amplification and detection of DNA and RNA targets.

Important: Use of unapproved supermixes can harm the instrument and void the warranty.

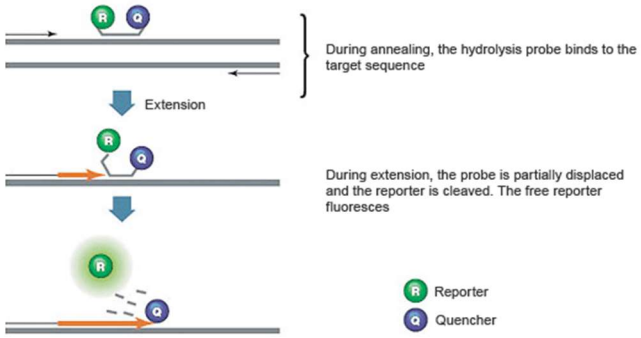
ddPCR Supermix	Description
Multiplex Supermix	For use in nucleic acid sample preparations to amplify and detect DNA targets in probe-based experiments involving multiple targets. **Note:** Bio-Rad recommends using ddPCR Multiplex Supermix for Cy5 and Cy5.5 probes assays.
Supermix for Probes (No dUTP)	For use in nucleic acid sample preparations, with sensitivity for the amplification and detection of DNA targets using hydrolysis probe-based assays.
Supermix for Probes	For use in sample preparation with uracil N-glucosylase (UNG) decontamination protocols to prevent the reamplification of carryover PCR products between experiments.
Supermix for Residual DNA Quantification	For use in residual DNA detection.
One-Step RT Advanced Kit for Probes	For use in absolute quantification of target RNA molecules. **Important:** Load only one (1) one-step RT plate, using the first slot in the QX ONE Droplet Digital PCR System plate Inbox. For more information, see Using the One Step RT ddPCR Advanced Kit for Probes.
EvaGreen® Supermix	For use in gene expression.

CT0478736 at CT0478802

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Claims	Exemplary Disclosure of Accused Product
	Hydrolysis probes are labeled with a fluorophore (e.g. a reporter) of the multiple fluorophores (e.g., FAM, HEX, Cy5, Cy5.5). *See, e.g.*:

BIO-RAD EX.1009.351

Claims	Exemplary Disclosure of Accused Product
	Hydrolysis (TaqMan) Probes Hydrolysis assays (TaqMan or 5' nuclease assays) include a sequence-specific, fluorescently labeled oligonucleotide probe in addition to a sequence-specific PCR primer. Hydrolysis assays exploit the 5' → 3' exonuclease activity of certain thermostable polymerases such as *Taq* or *Tth*. The hydrolysis probe is labeled with a fluorescent reporter at the 5' end and a quencher at the 3' end. Commonly used fluorescent reporter-quencher pairs are fluorescein (FAM), which emits green fluorescence, and Black Hole Quencher 1 dye.  **Fig. 2. Hydrolysis probes are hydrolyzed by *Taq* polymerase.** When the hydrolysis probe is intact, the fluorescence of the reporter is quenched due to its proximity to the quencher (Figure 2). The amplification reaction includes a combined annealing/extension step during which the probe hybridizes to the target, and the dsDNA-specific 5' → 3' exonuclease activity of *Taq* or *Tth* cleaves off the reporter. As a result, the reporter is separated from the quencher, resulting in a fluorescence signal that is proportional to the amount of amplified product in the sample. The main advantages of using hydrolysis probes are high specificity, a high signal-to-noise ratio, and the ability to perform multiplex reactions. The disadvantages are that the initial cost of the probe may be high, and the assay design may not be trivial. CT0481963 at CT0481965–CT0481966

BIO-RAD EX.1009.352

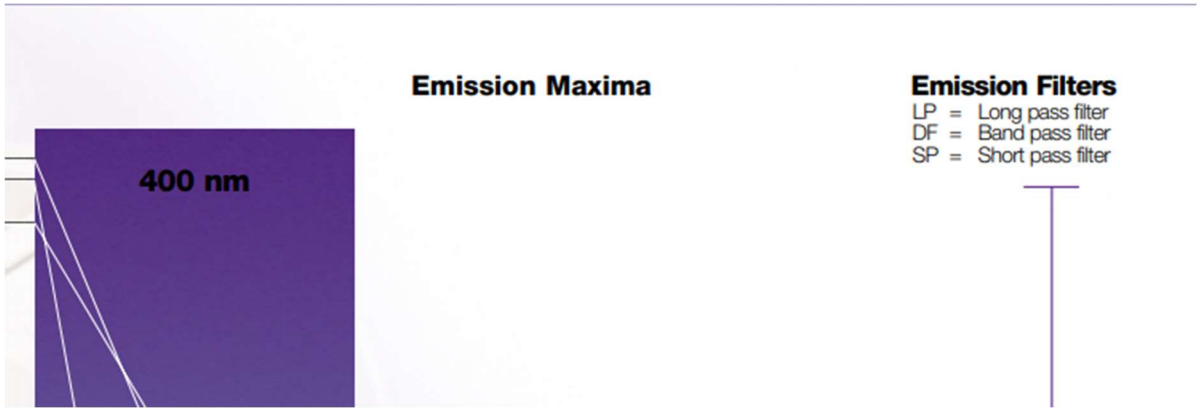
Claims	Exemplary Disclosure of Accused Product
	According to at least the above examples, the analyte-specific hybridization probes (e.g., hydrolysis probes) within the analyte-specific reagent mixture are labeled with a fluorophore (e.g., a reporter) of the multiple fluorophores (e.g., FAM, HEX, Cy5, Cy5.5), both literally and under the doctrine of equivalents.
[11.pre] 11. The system of claim 5, wherein	Caltech incorporates the discussion from claim 5, *supra,* in its entirety. As discussed therein, the QX ONE is a system comprising each and every element of claim 5, both literally and under the doctrine of equivalents.
[11.a] the detector comprises four or six band pass filters.	The multi-channel detector within QX ONE comprises four or six band pass filters, both literally and under the doctrine of equivalents. For example, the QX ONE detects electromagnetic waves at the emission wavelength of four fluorophores (e.g., FAM, HEX, Cy5, and Cy5.5). *See, e.g.*:

BIO-RAD EX.1009.353

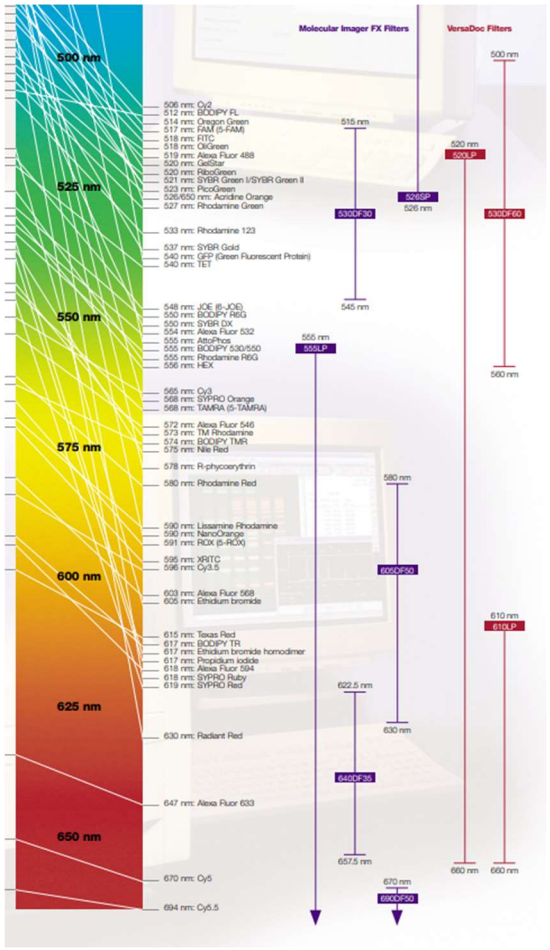
Claims	Exemplary Disclosure of Accused Product
	Instrument Specifications **Table 3. QX ONE Droplet Digital PCR System instrument specifications** CT0478736 at CT0478760

Element	Specifications
Size (W x D x H)	122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor
Weight	100 kg (220 lb) (includes the monitor but not reagents)
Capacity	5 plates
Samples	Up to 96 per plate
Partitions per sample	Approximately 20,000 droplets
Detection channels	4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel
Thermal gradient	Yes
Onboard computer	24 in, 1920 x 1080 resolution Holds data for approximately 100 plates
Time to results	6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol
Benchtop requirements	The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. **Important:** Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis.

BIO-RAD EX.1009.354

Claims	Exemplary Disclosure of Accused Product
	On information and belief, the QX ONE comprises at least four band-pass filters to filter received electromagnetic signals for signals with wavelengths near the emission wavelengths of the four fluorophores (e.g., FAM, HEX, Cy5, and Cy5.5). *See, e.g.* (band pass ("DF") filters utilized in Bio-Rad products listed with filter ranges around the emission wavelengths of fluorophores):  CT0479364 at CT0479364

BIO-RAD EX.1009.355

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364

BIO-RAD EX.1009.356

Claims	Exemplary Disclosure of Accused Product
	According to at least the above examples, the multi-channel detector within QX ONE comprises four or six band pass filters (e.g., four), both literally and under the doctrine of equivalents.
[13.pre] 13. The system of claim 5, wherein	Caltech incorporates the discussion from claim 5, *supra,* in its entirety. As discussed therein, the QX ONE is a system comprising each and every element of claim 5, both literally and under the doctrine of equivalents.
[13.a] the multi-channel detector comprises four or six photodetectors.	The multi-channel detector within QX ONE comprises four or six photodetectors, both literally and under the doctrine of equivalents. For example, the QX ONE detects electromagnetic waves at the emission wavelength of four fluorophores (e.g., FAM, HEX, Cy5, and Cy5.5). *See, e.g.*:

BIO-RAD EX.1009.357

Claims	Exemplary Disclosure of Accused Product
	Instrument Specifications **Table 3. QX ONE Droplet Digital PCR System instrument specifications** CT0478736 at CT0478760

Element	Specifications
Size (W x D x H)	122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor
Weight	100 kg (220 lb) (includes the monitor but not reagents)
Capacity	5 plates
Samples	Up to 96 per plate
Partitions per sample	Approximately 20,000 droplets
Detection channels	4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel
Thermal gradient	Yes
Onboard computer	24 in, 1920 x 1080 resolution Holds data for approximately 100 plates
Time to results	6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol
Benchtop requirements	The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. **Important:** Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis.

BIO-RAD EX.1009.358

Claims	Exemplary Disclosure of Accused Product
	On information and belief, the QX ONE comprises photodetectors at least capable of detecting electromagnetic signals with wavelengths near the emission wavelengths of the four fluorophores (e.g., FAM, HEX, Cy5, and Cy5.5). *See, e.g.* (droplet reader counting fluorescent positive droplets streamed in front of the detector, single file): Chapter 2 QX ONE Droplet Digital PCR System The QX ONE™ Droplet Digital PCR System combines droplet generation, thermal cycling, and droplet reading technologies into a single instrument, as described below: ■ The droplet generator partitions samples into approximately 20,000 uniform droplets. ■ The internal thermal cycler performs repeated heating and cooling processes to amplify one or more copies of a particular DNA segment in each droplet. ■ The droplet reader streams the droplets in single file, and then counts the fluorescent positive and negative droplets to calculate and quantify target DNA concentration. CT0478736 at CT0478758 According to at least the examples above, the multi-channel detector within QX ONE comprises four or six photodetectors (e.g., four photodetectors), both literally and under the doctrine of equivalents.
[14.pre] 14. The system of claim 5, wherein	Caltech incorporates the discussion from claim 5, *supra,* in its entirety. As discussed therein, the QX ONE is a system comprising each and every element of claim 5, both literally and under the doctrine of equivalents.

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Claims	Exemplary Disclosure of Accused Product
[14.a] the multi-channel detector comprises photodetectors that enable the detection of blue, green, and red fluorescent signals.	The multi-channel detector within QX ONE comprises photodetectors that enable the detection of blue, green, and red fluorescent signals, both literally and under the doctrine of equivalents. The QX ONE's photodetectors are capable of detecting blue, green, and red fluorescent signals. For example, the QX ONE detects electromagnetic waves at the emission wavelength of four fluorophores (e.g., FAM, HEX, Cy5, and Cy5.5). *See, e.g.*:

Instrument Specifications

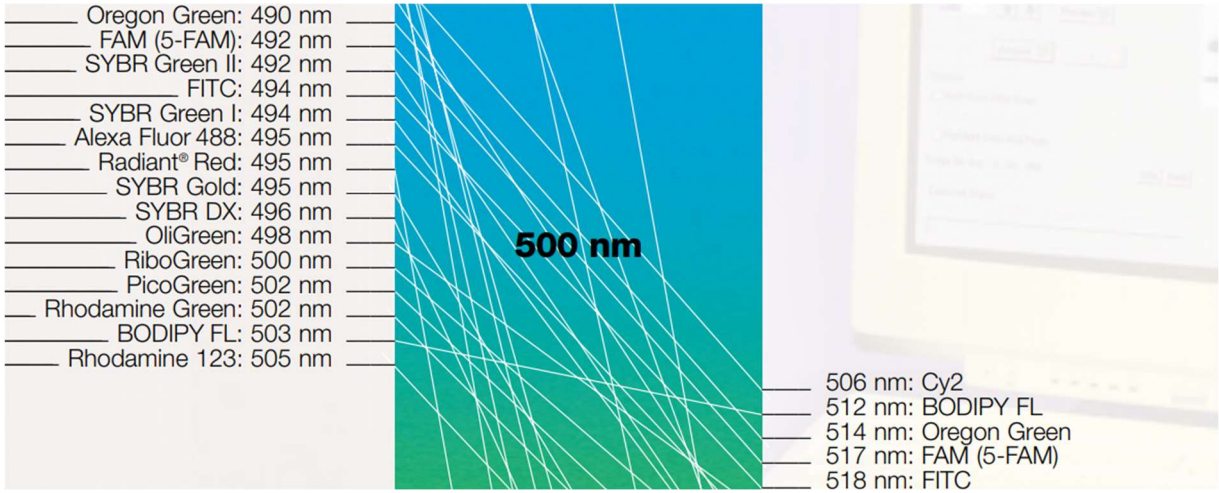
Table 3. QX ONE Droplet Digital PCR System instrument specifications

Element	Specifications
Size (W x D x H)	122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor
Weight	100 kg (220 lb) (includes the monitor but not reagents)
Capacity	5 plates
Samples	Up to 96 per plate
Partitions per sample	Approximately 20,000 droplets
Detection channels	4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel
Thermal gradient	Yes
Onboard computer	24 in, 1920 x 1080 resolution Holds data for approximately 100 plates
Time to results	6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol
Benchtop requirements	The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. Important: Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis.

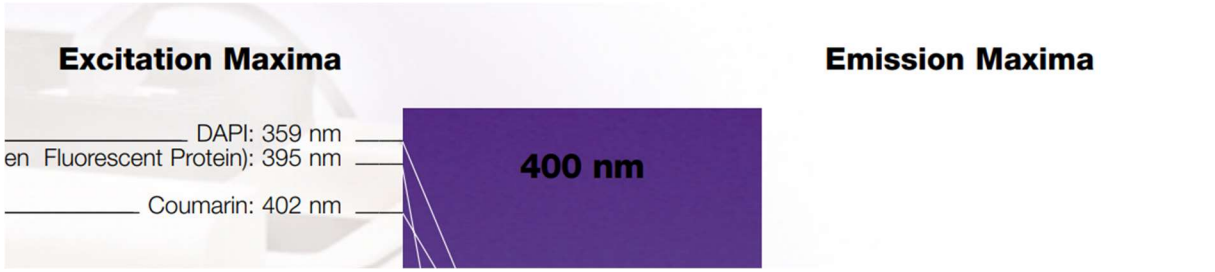
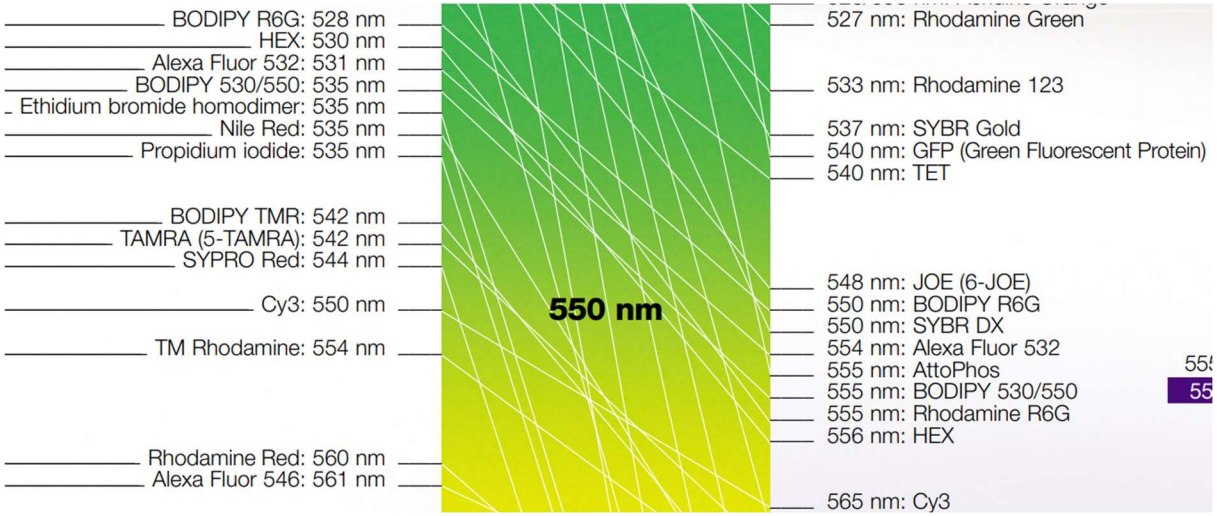
BIO-RAD EX.1009.360

Claims	Exemplary Disclosure of Accused Product
	CT0478736 at CT0478760 On information and belief, the QX ONE comprises photodetectors at least capable of detecting electromagnetic signals with wavelengths near the emission wavelengths of the four fluorophores (e.g., FAM, HEX, Cy5, and Cy5.5). *See, e.g.* (droplet reader counting fluorescent positive droplets streamed in front of the detector, single file): Chapter 2 QX ONE Droplet Digital PCR System The QX ONE™ Droplet Digital PCR System combines droplet generation, thermal cycling, and droplet reading technologies into a single instrument, as described below: ■ The droplet generator partitions samples into approximately 20,000 uniform droplets. ■ The internal thermal cycler performs repeated heating and cooling processes to amplify one or more copies of a particular DNA segment in each droplet. ■ The droplet reader streams the droplets in single file, and then counts the fluorescent positive and negative droplets to calculate and quantify target DNA concentration. CT0478736 at CT0478758 The FAM fluorophore is a green fluorophore. *See, e.g.*:

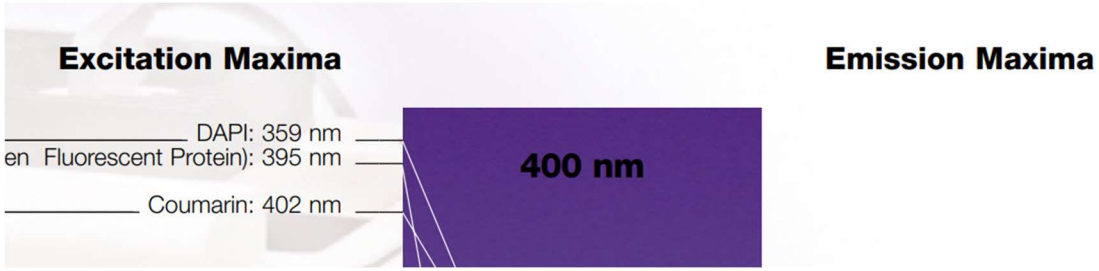
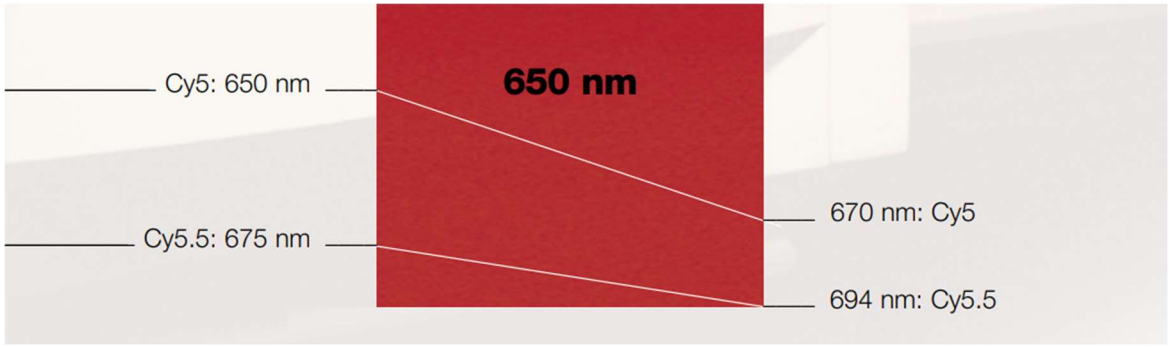
BIO-RAD EX.1009.361

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364  CT0479364 at CT0479364

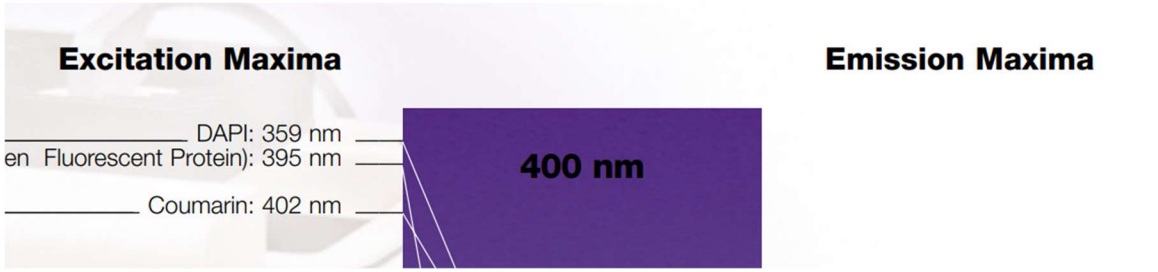
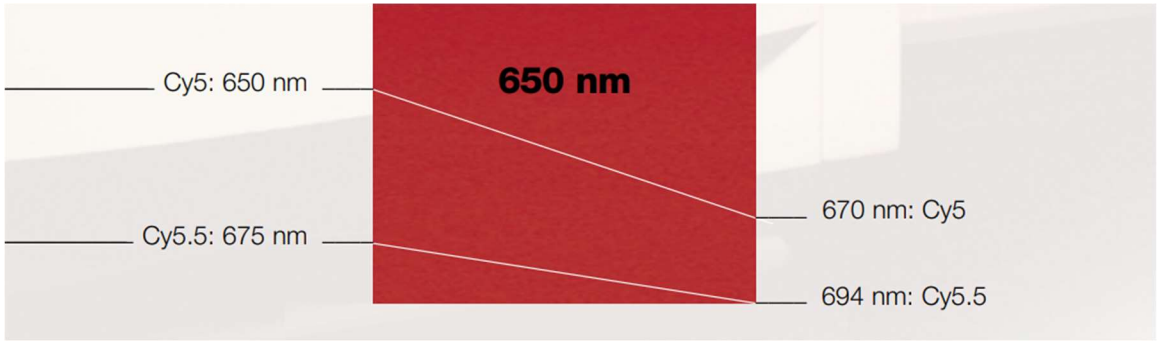
BIO-RAD EX.1009.362

Claims	Exemplary Disclosure of Accused Product
	The HEX fluorophore is a green and yellow fluorophore. *See, e.g.*:  CT0479364 at CT0479364  CT0479364 at CT0479364

BIO-RAD EX.1009.363

Claims	Exemplary Disclosure of Accused Product
	The Cy5 fluorophore is a red fluorophore. *See, e.g.*:  CT0479364 at CT0479364  CT0479364 at CT0479364

BIO-RAD EX.1009.364

Claims	Exemplary Disclosure of Accused Product
	The Cy5.5 fluorophore is a red fluorophore. *See, e.g.*:  CT0479364 at CT0479364  CT0479364 at CT0479364

BIO-RAD EX.1009.365

Claims	Exemplary Disclosure of Accused Product
	According to at least the examples above, the multi-channel detector within QX ONE comprises photodetectors that enable the detection of blue, green (e.g., FAM), and red (e.g., Cy5 and Cy5.5 wavelength) fluorescent signals, both literally and under the doctrine of equivalents.
[15.pre] 15. The system of claim 5, wherein	Caltech incorporates the discussion from claim 5, *supra,* in its entirety. As discussed therein, the QX ONE is a system comprising each and every element of claim 5, both literally and under the doctrine of equivalents.
[15.a] the multi-channel detector comprises photodetectors that enable the detection ranges selected from the group consisting of from about 483 nm to about 533 nm, from about 523 nm to about 568 nm, from about 558 nm to about 610 nm, from about 615 nm to about 670 nm, and any combinations thereof.	The multi-channel detector within QX ONE comprises photodetectors that enable the detection ranges selected from the group consisting of from about 483 nm to about 533 nm, from about 523 nm to about 568 nm, from about 558 nm to about 610 nm, from about 615 nm to about 670 nm, and any combinations thereof, both literally and under the doctrine of equivalents. For example, the QX ONE detects electromagnetic waves at the emission wavelength of fluorophores (e.g., FAM, HEX, and Cy5). *See, e.g.*:

BIO-RAD EX.1009.366

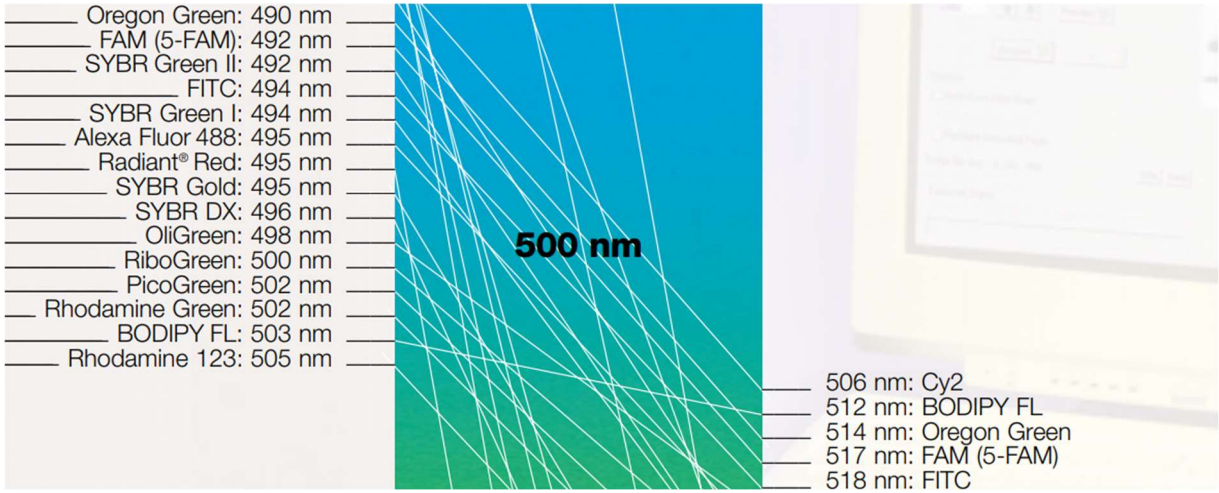
Claims	Exemplary Disclosure of Accused Product
	Instrument Specifications **Table 3. QX ONE Droplet Digital PCR System instrument specifications** CT0478736 at CT0478760

Element	Specifications
Size (W x D x H)	122 cm (48 in) x 66 cm (26 in) x 38 cm (15 in) 76 cm (30 in) top of monitor
Weight	100 kg (220 lb) (includes the monitor but not reagents)
Capacity	5 plates
Samples	Up to 96 per plate
Partitions per sample	Approximately 20,000 droplets
Detection channels	4 (FAM, HEX, Cy5, and Cy5.5), plus scatter channel
Thermal gradient	Yes
Onboard computer	24 in, 1920 x 1080 resolution Holds data for approximately 100 plates
Time to results	6.5 hours for the first plate, and approximately 4 hours for each plate thereafter, based on a standard 2-step ddPCR cycling protocol
Benchtop requirements	The following requirements apply to the benchtop or table on which the instrument will be installed. The benchtop or table must be 76 in W x 30 in D x 50 in H (193 cm x 76 cm x 126 cm), single benchtop rated for more than 250 lbs (114 kg) load. Ensure there is enough room above the instrument to ensure ergonomic safety. **Important:** Do not place instrument over multiple benchtop surfaces. Level within approximately 1° about each axis.

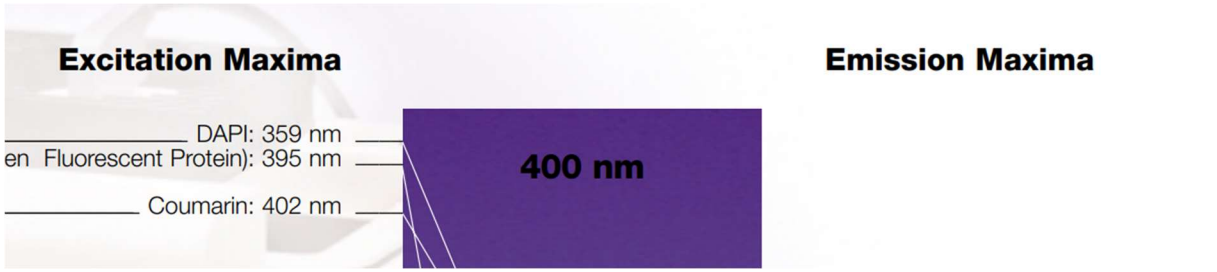
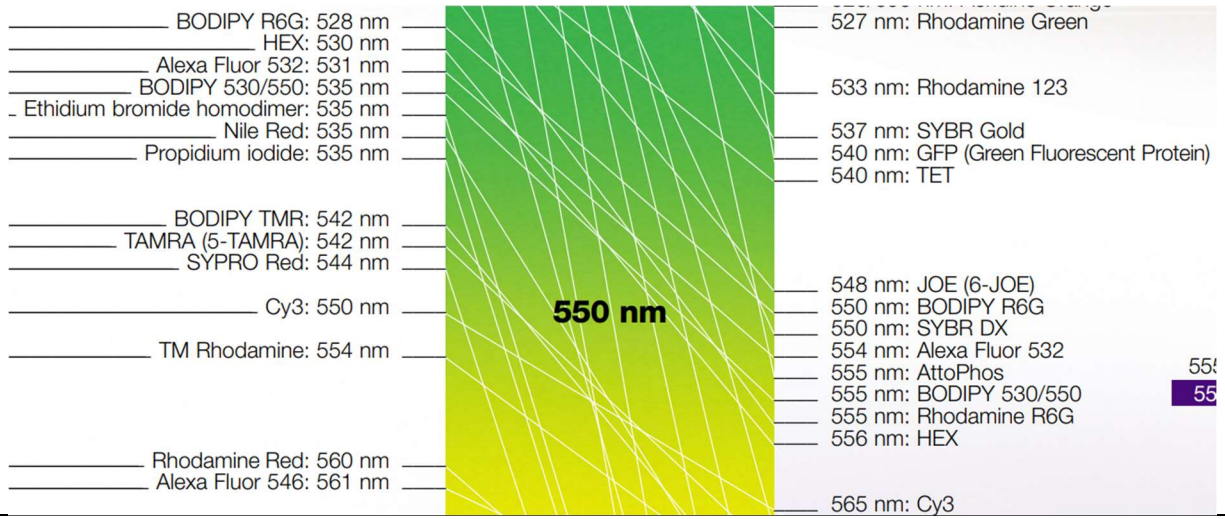
BIO-RAD EX.1009.367

Claims	Exemplary Disclosure of Accused Product
	On information and belief, the QX ONE comprises photodetectors at least capable of detecting electromagnetic signals with wavelengths near the emission wavelengths of the fluorophores (e.g., FAM, HEX, and Cy5). *See, e.g.* droplet reader counting fluorescent positive droplets streamed in front of the detector, single file: Chapter 2 QX ONE Droplet Digital PCR System The QX ONE™ Droplet Digital PCR System combines droplet generation, thermal cycling, and droplet reading technologies into a single instrument, as described below: ■ The droplet generator partitions samples into approximately 20,000 uniform droplets. ■ The internal thermal cycler performs repeated heating and cooling processes to amplify one or more copies of a particular DNA segment in each droplet. ■ The droplet reader streams the droplets in single file, and then counts the fluorescent positive and negative droplets to calculate and quantify target DNA concentration. CT0478736 at CT0478758 The FAM fluorophore has a maximum emission wavelength of from about 483 nm to about 533 nm (e.g., 517 nm). *See, e.g.*:

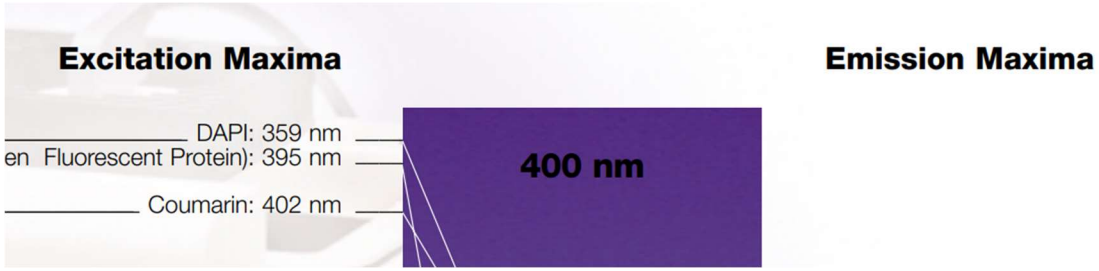
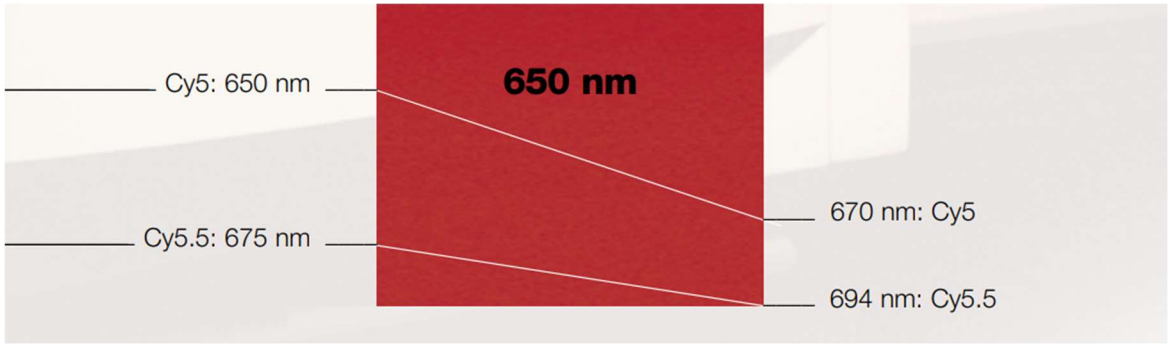
BIO-RAD EX.1009.368

Claims	Exemplary Disclosure of Accused Product
	 CT0479364 at CT0479364  CT0479364 at CT0479364

BIO-RAD EX.1009.369

Claims	Exemplary Disclosure of Accused Product
	The HEX fluorophore has a maximum emission wavelength of from about 523 nm to about 568 nm, (e.g., 556 nm). *See, e.g.*:  CT0479364 at CT0479364 

BIO-RAD EX.1009.370

Claims	Exemplary Disclosure of Accused Product
	CT0479364 at CT0479364 The Cy5 fluorophore has a maximum emission wavelength of from about 615 nm to about 670 nm (e.g., 670 nm). *See, e.g.*:  CT0479364 at CT0479364  CT0479364 at CT0479364

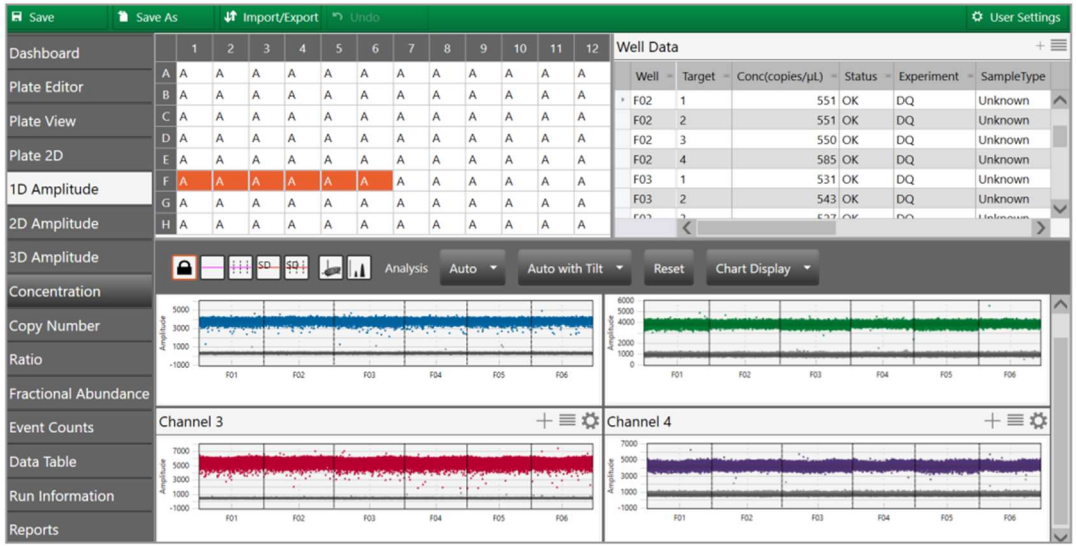
BIO-RAD EX.1009.371

Claims	Exemplary Disclosure of Accused Product
	The multi-channel detector within QX ONE comprises photodetectors that enable the detection ranges selected from the group consisting of from about 483 nm to about 533 nm (e.g., wavelengths near the FAM emission wavelength of 517 nm), from about 523 nm to about 568 nm (e.g., wavelengths near the HEX emission wavelength of 556 nm), from about 558 nm to about 610 nm, from about 615 nm to about 670 nm (e.g., wavelengths near the Cy5 emission wavelength of 670 nm), and any combinations thereof, both literally and under the doctrine of equivalents.
[18.pre] 18. The system of claim 5, further comprising	Caltech incorporates the discussion from claim 5, *supra,* in its entirety. As discussed therein, the QX ONE is a system comprising each and every element of claim 5, both literally and under the doctrine of equivalents.
[18.a] a display coupled to the processor controlled analyzer to visualize a plot of the first and second values.	The QX ONE comprises a display coupled to the processor controlled analyzer to visualize a plot of the first and second values, both literally and under the doctrine of equivalents. For example, QX ONE comprises an integrated touch screen, which includes a display for displaying analysis data. *See, e.g.*:

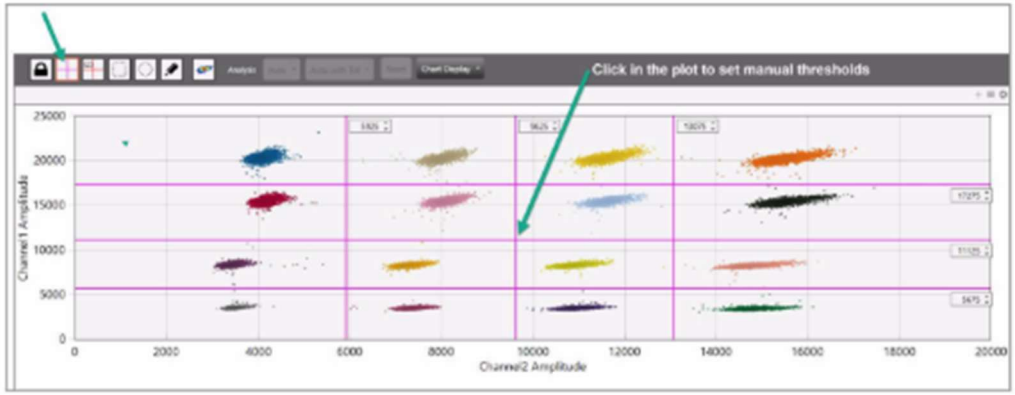
BIO-RAD EX.1009.372

Claims	Exemplary Disclosure of Accused Product
	QX ONE Droplet Digital PCR System Components The QX ONE Droplet Digital PCR System ships with the following items: ■ The instrument, which performs the sequential ddPCR processes ■ Attached touch screen, which is a computer that displays the software interface and connects to the instrument ■ QX ONE Software, which is installed on the instrument touch screen computer, and provides a user interface to □ Control the instrument □ Set up assays □ Collect analysis data □ Recover plates from run failures □ Store analysis data □ Display analysis data CT0478736 at CT0478759 The QX ONE analysis data displays provide visual plots of first values (e.g., channel intensity values) and second values (e.g., channel wavelength values). *See e.g.*:

BIO-RAD EX.1009.373

Claims	Exemplary Disclosure of Accused Product
	1D Amplitude Use the 1D Amplitude analysis window to view droplet amplitudes for each channel, in selection order, for each well selected in the Well Selector.  CT0478876 at CT0478985

BIO-RAD EX.1009.374

Claims	Exemplary Disclosure of Accused Product
	 CT0478876 at CT0479047 According to at least the examples above, the QX ONE comprises a display coupled to the processor controlled analyzer to visualize a plot of the first (e.g., channel intensity) and second (e.g., channel wavelength) values, both literally and under the doctrine of equivalents.

BIO-RAD EX.1009.375