

Appendix A-1: Infringement Claim Chart for U.S. Patent No. 10,068,051 and QX600 Droplet Digital PCR System

Use of Bio-Rad's QX600 Droplet Digital PCR System ddPCR ("QX600") infringes Claims 1-9 (the "Asserted Claims") of U.S. Patent No. 10,068,051 ("the '051 Patent"). The accused QX600 includes both 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 the QX600. Reference to QX600 will be understood to refer to both versions of the system.

As non-limiting examples, Plaintiffs identify where and how use of the Accused Product performs each and every step of the below claimed method. Elements from other charts may be used in combination with the below to perform the steps of the claimed method, and Plaintiffs incorporate all other '051 Charts here by reference.

On information and belief, the QX600 is used with other devices, accessories, and components to practice the claimed methods of the '051 Patent. See <https://www.bio-rad.com/en-us/product/qx600-droplet-digital-pcr-system?ID=b07d12ac-0585-fc4c-a586-3ddf20d5c4a0> and <https://www.bio-rad.com/en-us/product/qx600-autodg-droplet-digital-pcr-system?ID=0d9e49a4-cf92-f9a9-5779-ef412180f1ed>. 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
 - o PrimePCR ddPCR Assays,
 - o ddPCR Multiplex Supermix,
 - o ddPCR Supermixes for Probes (No dUTP),
 - o ddPCR Supermix for Probes,
 - o One-Step RT-ddPCR Advanced Kit for Probes,
 - o ddPCR Buffer Control for Probes,
 - o Residual DNA Quantification Supermixes,
 - o QX200 ddPCR EvaGreen Supermix,
 - o QX200 Buffer Control for EvaGreen, and
 - o ddPCR Library Quantification Kit for Illumina TruSeq;
- Consumables, such as

- o ddPCR 96-Well Plates,
- o Droplet Generation Oil for Probes,
- o QX200 Droplet Generation Oil for EvaGreen,
- o Droplet Reader Oil,
- o DG8 Cartridges and DG8 Gaskets,
- o DG8 Cartridge Holders,
- o pierceable foil such as the PCR Plate Heat Seal,
- o DG32 Cartridges for AutoDG,
- o Pipet Tips for AutoDG,
- o Pipet Tip Waste Bin for AutoDG;
- PrimePCR Primers, Assays and Arrays;
- Each of the assays identified in these Infringement Contentions; and/or
- Any other processes, procedures, devices, and/or system components used to facilitate the performance of the steps of the Asserted Claims.

See <https://www.bio-rad.com/en-us/product/qx600-droplet-digital-pcr-system?ID=b07d12ac-0585-fc4c-a586-3ddf20d5c4a0>;
<https://www.bio-rad.com/en-us/product/qx600-autodg-droplet-digital-pcr-system?ID=0d9e49a4-cf92-f9a9-5779-ef412180f1ed>.

Bio-Rad directly infringes the Asserted Claims by using the QX600 alone and/or with the above-listed devices, software, accessories, and/or components to perform each and every step of the claim methods below. By way of non-limiting example, Bio-Rad uses the QX600 in connection with (but not exclusively with) the various assays identified in these Infringement Contentions to practice each and every step of the Asserted Claims.

Bio-Rad also indirectly infringes the Asserted Claims by providing the QX600 alone and/or with the accompanying QX Manager Software. Bio-Rad also induces customers and/or end users to use the QX600 and/or QX Manager Software to infringe by inducing them to use these products in a way that performs each step 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, such customers and/or end users use the QX600 in connection with (but not exclusively with) the various assays identified in these Infringement Contentions to practice each and every step of the Asserted Claims.

Bio-Rad also contributes to the infringement of customers and/or end-users by providing the QX600 and/or the QX Manager to the same who then perform each step 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 QX600 in connection with

(but not exclusively with) the various assays identified in these Infringement Contentions to practice each and every step 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 QX Manager Software such that Bio-Rad induces infringe 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).

On information and belief, to the extent Bio-Rad performs the steps of the Asserted Claims outside the United States and subsequently imports, offers to sell, sells, and/or uses the results of the claimed methods within the United States, Bio-Rad infringes in violation of 35 U.S.C. § 271(g).

Plaintiffs allege infringement both literally and under the doctrine of equivalents.

Claims	Exemplary Disclosure of Accused Product
[1-PRE] 1. A method of detecting the presence or absence of at least seven polynucleotide analytes, in any combination of presence or absence, in a single sample solution volume, the method comprising:	To the extent the preamble is found to be limiting, the QX600 Droplet Digital PCR System is used to perform a method of detecting the presence or absence of at least seven polynucleotide analytes, in any combination of presence or absence, in a single sample solution volume, both literally and under the doctrine of equivalents. For example:

Claims	Exemplary Disclosure of Accused Product
	QX600 Droplet Digital PCR System The QX600™ Droplet Digital™ PCR System enables advanced six-color multiplexing, allowing clear discrimination of multiple targets with assays that are cross-compatible with the QX200™ Droplet Digital PCR System. The QX600 System is designed for researchers who need to quantify multiple targets with high accuracy, reproducibility, and sensitivity. Read More

Claims	Exemplary Disclosure of Accused Product
	Overview The QX600 Droplet Digital PCR System (ddPCR™) offers advanced six-color multiplexing and absolute quantification of nucleic acid, while delivering the best-in-class performance you have come to expect from Bio-Rad's ddPCR systems. Benefits of the QX600 Droplet Digital PCR System The QX600 Droplet Reader offers users: • Sensitive multiplexing • Six-color detection capability (FAM, HEX, Cy5, Cy5.5, ROX, and ATTO 590) • Quantification of up to 12 targets in a single well • Absolute quantification with 0.1% or better sensitivity • Sensitive and precise gene expression multiplexing • Accurate and reproducible results • Confidence in your results bolstered by a system qualified by standards from National Measurement Institute (NMI), Australia • Sets the standard for reproducibility as demonstrated in numerous studies • Powerful software • User-friendly analysis • Audit trails and tools for U.S. FDA 21 CFR Part 11 compliance with premium edition • Assay protocol files for automated data analysis and reporting with premium edition • Portfolio compatibility • Cross-compatibility with QX200 reagents, assays, and consumables • Extensive menu of pre-existing ddPCR assays • System capable of running both TaqMan® and EvaGreen chemistries See https://www.bio-rad.com/en-us/product/qx600-droplet-digital-pcr-system?ID=b07d12ac-0585-fc4c-a586-3ddf20d5c4a0.

Claims	Exemplary Disclosure of Accused Product
	SENSITIVE MULTIPLEXING ▪ 6-color detection capability (FAM/EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590) ▪ Quantification of 12 targets in a single well ▪ Absolute quantification with 0.1% or better sensitivity BIO_CC00008190 at BIO_CC00008191 The ability to quantify 12 targets with 6 colors must include the steps of identifying the presence or absence of at least 7 of those targets. SIX-COLOR DETECTION CAPABILITY WITH HIGH ACCURACY, PRECISION, AND REPRODUCIBILITY The QX600 ddPCR System offers six-color multiplexing for the clear discrimination of multiple targets and more accurate and reproducible quantification with six-color detection capability (FAM/EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590). It has been qualified by traceable material from the National Measurement Institute of Australia. BIO_CC00008190 at BIO_CC00008192 The system allows for clear discrimination of multiple targets and more accurate and reproducible quantification, which allows it to detect the presence or absence of polynucleotide analytes with high accuracy, precision, and reproducibility. Droplet Digital™ PCR (ddPCR™) is a digital polymerase chain reaction method based on water-oil emulsion droplet technology. ddPCR uses a combination of microfluidics and proprietary surfactant chemistries to divide each sample into water-in-oil droplets. The technology uses reagents and workflows similar to those used for most standard TaqMan probe-based assays, and provides absolute quantification of nucleic acid target sequences by counting nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions. BIO_CC00007427 at BIO_CC00007449 The QX600 system provides absolute quantification of nucleic acid target sequences detecting the presence or absence of polynucleotide analytes, such as by counting nucleic acid molecules encapsulated in discrete volumetrically defined water-in-oil droplet partitions.

Claims

Exemplary Disclosure of Accused Product

The Automated Droplet Generator is compatible with QX600 Droplet Digital PCR Systems.

Compatible ddPCR Process Instruments

Along with the QX600 Droplet Reader, you can purchase the droplet generators shown below from Bio-Rad.

QX200 Droplet Generator



Automated Droplet Generator (left)



QX600 Droplet Reader



Important: A thermal cycler, which can be purchased separately, is required to perform a full ddPCR cycle. For information on additional instruments, accessories, and consumables, see [Appendix D, Ordering Information](#).

BIO_CC00007427 at BIO_CC00007458

The QX600 AutoDG Droplet Digital PCR System (ddPCR) offers workflow automation, delivers advanced six-color multiplexing, and absolute quantification of nucleic acid without sacrificing the best-in-class performance you have come to expect from Bio-Rad's ddPCR systems.

The AutoDG Instrument eliminates user-to-user variability, allowing you to fully exploit the precision of ddPCR technology by providing more consistent droplet counts across users and plates. The AutoDG Instrument also minimizes hands-on time — it takes less than 5 minutes to load the instrument, and no supervision or user activity is required during droplet generation.

Benefits of the QX600 AutoDG Droplet Digital PCR System

The Automated Droplet Generator offers:

- A simplified ddPCR workflow
- Generates droplets for up to 96 ddPCR samples in under 45 minutes
- Compatibility with QX200™ reagents and consumables
- Guided setup and loading with user-friendly touch-screen interface
- Run-to-run flexibility with user-definable plate layout in 8-well increments
- Reduced contamination risk with HEPA-filtered enclosure allowing the system to be placed in any lab environment

See <https://www.bio-rad.com/en-us/product/qx600-autodg-droplet-digital-pcr-system?ID=0d9e49a4-cf92-f9a9-5779-ef412180f1ed>

Claims

Exemplary Disclosure of Accused Product

ddPCR System and Instrument Packages		
Table 46. 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
QX200 AutoDG™ Droplet Digital PCR System	Includes the AutoDG, QX200 Droplet Reader, computer, and QX Manager Software Standard Edition	1864100
QX600 AutoDG Droplet Digital PCR System	Includes the AutoDG, QX600 Droplet Reader, computer, QX Manager Software Standard Edition, and consumables	17008371
Automated Droplet Generator	Includes the AutoDG (1), cooling block accessory (1), oil purge reservoir (1), and power cord (1)	1864101

BIO_CCODE00007427 at BIO_CCODE00007633

Automated Droplet Generator #1864101



Overview

AutoDG Instrument for automated droplet generation of up to 96 samples, for use with the QX600/QX200 Droplet Digital PCR Systems

List Price: **Inquire**

Your Price: [Log in](#) to see your price

Quantity: [Add to Cart](#) [Add to Quote](#) [Add to Hot List](#)

See <https://www.bio-rad.com/en-us/sku/1864101-automated-droplet-generator?ID=1864101>

Claims	Exemplary Disclosure of Accused Product
[1a.1] a) providing a sample solution volume comprising or potentially comprising, at least one of at least seven polynucleotide analytes,	The QX600 Droplet Digital PCR System is used to perform ways of providing a sample solution volume comprising or potentially comprising, at least one of at least seven polynucleotide analytes, both literally and under the doctrine of equivalents. An exemplary description of the ddPCR Workflow is described in the QX600 Instruction Manual, which also describes a process for sample solution provision: 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. BIO_CC00007427 at 7450; <i>see also</i> BIO_CC00007643 at 7666. Samples may be provided by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermixes. <i>See id.</i> Samples may be further provided using a droplet generator such as the AutoDG or QX200 Droplet Generator. <i>See, e.g.,</i> BIO_CC00006235 at -6271 through -6289; BIO_CC00008417-8418; BIO_CC00006175 at -6203 through 7207. Samples may be further provided by via a thermal cycler. Two exemplary thermal cyclers offered by Bio-Rad include the PTC Tempo Thermal Cycler and the C1000 Touch Thermal Cycler.

- Programmable thermal gradient — for optimization of annealing temperatures
- Large touch screen — for streamlined protocol programming and editing
- Heated and automated lid — for use with various vessels, sealer types, and for integration with automation robotics
- Expanded connectivity — WiFi, ethernet, and USB
- Cloud connectivity: Create and send protocols to the PTC Tempo Thermal Cycler from the BR.io cloud platform
- Network storage drive access for excellent data management

Claims	Exemplary Disclosure of Accused Product
	https://www.bio-rad.com/en-us/product/c1000-touch-thermal-cycler?ID=LGTW9415; https://www.bio-rad.com/en-us/sku/12015392-ptc-tempo-deepwell-thermal-cycler?ID=12015392. During the thermal cycling, a plate sealer such as the PX1 PCR Plate Sealer may optionally be used: Description Use the PX1 PCR Plate Sealer for consistent, uniform sealing across the entire plate, minimizing evaporation during cycling. The small-footprint heat sealer is compatible with a wide range of PCR plates and different types of films and foils. Features and Benefits • Ready to seal in less than 3 min • Support block holds both 96- and 384 well plates • Motorized drawer for insertion of plate and seal • Touch-screen interface allowing single button modification of temperature and time • Easy access to previously stored sealing protocols The intuitive touch-screen interface of the PX1 PCR Plate Sealer shows instrument status, platen temperature, protocol name, set temperature, and time. Sealing protocols can be stored and easily edited. The sealer can be programmed to automatically shut down after a user-defined period of idle time. Touching the display after shutdown will reactivate the display and the sealer will begin heating to the last set temperature. The semi-automated PX1 PCR Plate Sealer provides fast, consistent sealing, removing human variability and increasing reproducibility. Uniform sealing over the whole plate is particularly important for low-volume samples where small amounts of evaporation can have significant effect. In qPCR experiments, differential evaporation from wells can lead to large standard deviations in the number of quantification cycles (Cq). Product Components • Heat sealing instrument • Plate support block for both 96- and 384-well plates • Sealing frame Related Products • Optically Clear Heat Seals, for PCR and real-time PCR applications (1814030) • Permanent Clear Heat Seals, nonpeelable, nonpierceable clear seals for PCR, water bath cycling, and sample disposal (1814035) • Pierceable Foil Heat Seals for PCR and QX200™ Droplet Digital™ PCR System applications (1814040) • Peelable Foil Heat Seals laminate/foil peelable seals for PCR and sample storage (1814045) https://www.bio-rad.com/en-us/sku/1814000-px1-pcr-plate-sealer?ID=1814000. Samples may be also provided via a well plate in connection with the QX600 Droplet Reader. <i>See generally</i> BIO_CC00007427, BIO_CC00007643, BIO_CC00008141, BIO_CC00008190, BIO_CC00008194, BIO_CC00008198, and

Claims	Exemplary Disclosure of Accused Product
	BIO_CC00008202. Exemplary processes for providing samples is described at BIO_CC00007427 at 7477-7493 and BIO_CC00007643 at 7691-7711. The samples provided in connection with the QX600 comprise at least of at least seven polynucleotide analytes (referred to as “targets”). For example: SENSITIVE MULTIPLEXING ▪ 6-color detection capability (FAM/EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590) ▪ Quantification of 12 targets in a single well ▪ Absolute quantification with 0.1% or better sensitivity BIO_CC00008190 at 8191. Droplet Digital™ PCR (ddPCR™) is a digital polymerase chain reaction method based on water-oil emulsion droplet technology. ddPCR uses a combination of microfluidics and proprietary surfactant chemistries to divide each sample into water-in-oil droplets. The technology uses reagents and workflows similar to those used for most standard TaqMan probe-based assays, and provides absolute quantification of nucleic acid target sequences by counting nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions. BIO_CC00007427 at 7449. The QX600 system requires dividing each sample into a sample solution volume (e.g., a water-in-oil droplet) with target and background DNA randomly distributed into the droplets and allowing for quantification of at least seven targets in a single well:

Claims

Exemplary Disclosure of Accused Product

Assay Types and Fluorophores

QX Manager Software offers the assay methods explained in [Table 19](#).

Table 19. Assay types

Assay type	Description
Single target per channel	Method assuming up to six probe colors (FAM/EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590) and up to six targets per channel. This is the default assay type, with the maximum six rows displayed. You can delete up to three rows.
Amplitude multiplex	Method to increase multiplexing up to twelve 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 three 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. Group 3 has 1 target in ROX, 1 target in ATTO 590, and one target in both groups. You can delete a group, but not the individual targets within the group. Note: The channels within each 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 <u>and</u> HEX/VIC and one target in FAM <u>or</u> 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 <u>or</u> Cy5.5. You can delete a group, but not the individual targets in the group. Note: The channels within each group are not interchangeable therefore FAM is always paired with HEX/VIC and Cy5 with Cy5.5.

Claims

Exemplary Disclosure of Accused Product

Table 19. Assay types, continued

Assay type	Description
Double drop-off	This assay type is available only for the DOF experiment type, and allows three groups of three targets each. The first group appears by default, and looks for one target in FAM <u>and</u> in HEX/VIC (target type reference) one target in FAM (target type unknown), and one target in HEX/VIC (target type unknown). You can add the second group to look for one target in Cy5 <u>and</u> Cy5.5 (target type reference, with two reference copies), one target in Cy5 (target type unknown), and one target in Cy5.5 (target type unknown). You can also add a third group to look for one target in ROX <u>and</u> ATTO 590 (target type reference), one target in ROX (target type unknown), and one target in ATTO 590 (target type unknown). By default, all reference copies are set to 2. The channels are static for each group. (Channel 1 FAM is always paired with Channel 2 HEX/VIC, Channel 3 Cy5 is always paired with Channel 4 Cy5.5, and Channel 5 ROX is always paired with Channel 6 ATTO 590.

The experiment type you select determines the available assay methods. [Table 20](#) displays the sample type associated with each assay method.

Table 20. Assay types by experiment type

Assay type	Experiment type						
	DQ	CNV	MUT	DOF	GEX	RDQ	RED
Single target per channel	✓	✓	✓		✓	✓	✓
Amplitude multiplex	✓	✓	✓		✓	✓	✓
Probe mix triplex	✓	✓	✓		✓	✓	✓
Advanced classification	✓						✓
Basic drop-off				✓			
Double drop-off				✓			

Claims	Exemplary Disclosure of Accused Product																																																																																																												
	QX Manager Software automatically populates the fluorophore in each channel for each target but you can change the default selections. Use Table 21 to select from the fluorophore options for each assay method listed. Table 21. Fluorophore options <table><tr><th>Assay method</th><th>Signal Ch1</th><th>Signal Ch2</th><th>Signal Ch3</th><th>Signal Ch4</th><th>Signal Ch5</th><th>Signal Ch6</th></tr><tr><td rowspan="3">Single target per channel, for 1 to 6 targets</td><td>FAM</td><td>HEX</td><td>Cy5</td><td>Cy5.5</td><td>ROX</td><td>ATTIO590</td></tr><tr><td>EvaGreen®</td><td>VIC</td><td>None</td><td>None</td><td>None</td><td>None</td></tr><tr><td>None</td><td>None</td><td></td><td></td><td></td><td></td></tr><tr><td rowspan="5">Amplitude multiplex, for 1 to 12 targets</td><td>FAM Hi</td><td>HEX Hi</td><td>Cy5 Hi</td><td>Cy5.5 Hi</td><td>ROX Hi</td><td>ATTO 590 Hi</td></tr><tr><td>FAM Lo</td><td>HEX Lo</td><td>Cy 5 Lo</td><td>Cy 5.5 Lo</td><td>ROX Lo</td><td>ATTO 590 Lo</td></tr><tr><td>EvaGreen® Hi</td><td>VIC Hi</td><td>None</td><td>None</td><td>None</td><td>None</td></tr><tr><td>EvaGreen® Lo</td><td>VIC Lo</td><td></td><td></td><td></td><td></td></tr><tr><td>None</td><td>None</td><td></td><td></td><td></td><td></td></tr><tr><td rowspan="3">Probe mix triplex, for 9 targets</td><td>FAM</td><td>HEX</td><td>Cy5</td><td>Cy5.5</td><td>ROX</td><td>ATTO 590</td></tr><tr><td>None</td><td>VIC</td><td>None</td><td>None</td><td>None</td><td>None</td></tr><tr><td></td><td>None</td><td></td><td></td><td></td><td></td></tr><tr><td rowspan="4">Advanced classification</td><td>FAM</td><td>HEX</td><td>Cy5</td><td>Cy5.5</td><td>ROX</td><td>ATTO 590</td></tr><tr><td>+1-10</td><td>1 to 10</td><td>1 to 10</td><td>1 to 10</td><td>1 to 10</td><td>1 to 10</td></tr><tr><td>EvaGreen®</td><td>VIC</td><td>None</td><td>None</td><td>None</td><td>None</td></tr><tr><td>1 to 10</td><td>1 to 10</td><td></td><td></td><td></td><td></td></tr><tr><td></td><td>None</td><td>None</td><td></td><td></td><td></td><td></td></tr></table> BIO_CC00007427 at 7505-7507; <i>see also</i> BIO_CC00007643.	Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4	Signal Ch5	Signal Ch6	Single target per channel, for 1 to 6 targets	FAM	HEX	Cy5	Cy5.5	ROX	ATTIO590	EvaGreen®	VIC	None	None	None	None	None	None					Amplitude multiplex, for 1 to 12 targets	FAM Hi	HEX Hi	Cy5 Hi	Cy5.5 Hi	ROX Hi	ATTO 590 Hi	FAM Lo	HEX Lo	Cy 5 Lo	Cy 5.5 Lo	ROX Lo	ATTO 590 Lo	EvaGreen® Hi	VIC Hi	None	None	None	None	EvaGreen® Lo	VIC Lo					None	None					Probe mix triplex, for 9 targets	FAM	HEX	Cy5	Cy5.5	ROX	ATTO 590	None	VIC	None	None	None	None		None					Advanced classification	FAM	HEX	Cy5	Cy5.5	ROX	ATTO 590	+1-10	1 to 10	1 to 10	1 to 10	1 to 10	1 to 10	EvaGreen®	VIC	None	None	None	None	1 to 10	1 to 10						None	None				
Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4	Signal Ch5	Signal Ch6																																																																																																							
Single target per channel, for 1 to 6 targets	FAM	HEX	Cy5	Cy5.5	ROX	ATTIO590																																																																																																							
	EvaGreen®	VIC	None	None	None	None																																																																																																							
	None	None																																																																																																											
Amplitude multiplex, for 1 to 12 targets	FAM Hi	HEX Hi	Cy5 Hi	Cy5.5 Hi	ROX Hi	ATTO 590 Hi																																																																																																							
	FAM Lo	HEX Lo	Cy 5 Lo	Cy 5.5 Lo	ROX Lo	ATTO 590 Lo																																																																																																							
	EvaGreen® Hi	VIC Hi	None	None	None	None																																																																																																							
	EvaGreen® Lo	VIC Lo																																																																																																											
	None	None																																																																																																											
Probe mix triplex, for 9 targets	FAM	HEX	Cy5	Cy5.5	ROX	ATTO 590																																																																																																							
	None	VIC	None	None	None	None																																																																																																							
		None																																																																																																											
Advanced classification	FAM	HEX	Cy5	Cy5.5	ROX	ATTO 590																																																																																																							
	+1-10	1 to 10	1 to 10	1 to 10	1 to 10	1 to 10																																																																																																							
	EvaGreen®	VIC	None	None	None	None																																																																																																							
	1 to 10	1 to 10																																																																																																											
	None	None																																																																																																											
[1a.2] and comprising at least seven hybridization probes, each hybridization probe corresponding to	The QX600 is used to perform a method using samples with at least seven hybridization probes, each hybridization probe corresponding to one of the at least seven polynucleotide analytes, both literally and under the doctrine of equivalents. When the QX600 is used, the samples provided comprise at least seven hybridization probes with each hybridization probe corresponding to one of at least seven polynucleotide analytes, for example:																																																																																																												

Claims	Exemplary Disclosure of Accused Product
one of the at least seven polynucleotide analytes,	PORTFOLIO COMPATIBILITY - Same assays, reagents, and consumables as QX200 Droplet Digital PCR System - Extensive menu of existing ddPCR Assays for EvaGreen® and hydrolysis probe chemistry BIO_CC00008190-8191. Chapter 1 Introduction to Droplet Digital PCR Droplet Digital™ PCR (ddPCR™) is a digital polymerase chain reaction method based on water-oil emulsion droplet technology. ddPCR uses a combination of microfluidics and proprietary surfactant chemistries to divide each sample into water-in-oil droplets. The technology uses reagents and workflows similar to those used for most standard TaqMan probe-based assays, and provides absolute quantification of nucleic acid target sequences by counting nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions. ddPCR is highly effective in the following areas: Absolute quantification — ddPCR provides a concentration of target DNA copies per input sample without the need for running standard curves, making this technique ideal for target DNA measurements, viral load analysis, and microbial quantification. Genomic alterations such as gene copy number variation (CNV) — CNVs result in too few or too many dosage-sensitive genes responsible for phenotypic variability, complex behavioral traits, and disease. ddPCR enables measurement of 1.2x differences in gene copy number. Detection of rare sequences — researchers must amplify single genes in a complex sample, such as a few tumor cells in a wild-type background. ddPCR is sensitive enough to detect rare mutations or sequences. Gene expression and microRNA analysis — ddPCR provides stand-alone absolute quantification of expression levels, especially low-abundance microRNAs, with sensitivity and precision. Next-generation sequencing (NGS) — ddPCR quantifies NGS sample library preparations to increase sequencing accuracy and reduce run repeats. Validate sequencing results such as single nucleotide polymorphisms or copy number variations with absolute quantification. Single cell analysis — the high degree (10-fold to 100-fold) of cell-cell variation in gene expression and genomic content among homogeneous post-mitotic, progenitor, and stem cell populations drives a need for analysis from single cells. ddPCR enables low copy number quantification and gene expression of individual cells. Genome edit detection — ddPCR enables fast, precise, and cost-effective assessment of HDR (Homology directed repair) and NHEJ (non-homologous end joining) generated by CRISPR-Cas9 or other genome editing tools.

Claims

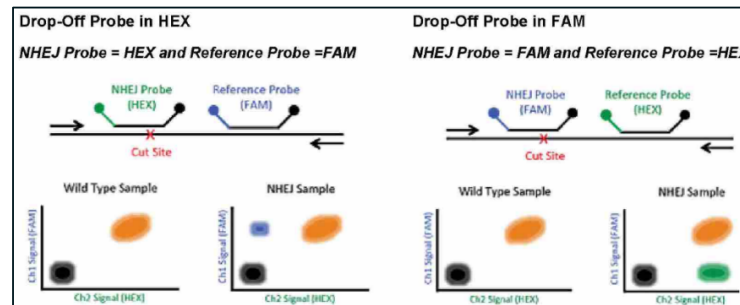
Exemplary Disclosure of Accused Product

ddPCR has the following benefits for nucleic acid quantification:

- **Unparalleled precision** — The massive sample partitioning afforded by ddPCR enables small fold differences in target DNA sequence between samples to be reliably measured.
- **Increased signal-to-noise ratio** — High-copy templates and background are diluted, effectively enriching template concentration in target-positive partitions. This allows for the sensitive detection of rare targets and enables a $\pm 10\%$ precision in quantification.
- **Removal of PCR efficiency bias** — Error rates are reduced by removing the amplification efficiency reliance of qPCR, enabling accurate quantification of targets to near zero.
- **Simplified quantification** — There is no requirement for a standard curve for absolute quantification.

BIO_CCODE00007427 at 7449-7450; *see also* BIO_CCODE00007643.

The QX600 Droplet Digital PCR System uses kits that use hybridization probes for the detection of targets. The following provides an example disclosure for how the hybridization probes correspond to one of the targets. For example:



BIO_CCODE00007427 at 7618; *see also* BIO_CCODE00007643; *see* additional examples in Appendix A of the user guides.

As shown in the example figure above, each probe corresponds to one of the at least seven targets (i.e., polynucleotide analytes).

Claims	Exemplary Disclosure of Accused Product												
	The QX600 user guide provides various additional examples which are incorporated here by reference. <i>See generally</i> BIO_CCODE00007427, BIO_CCODE00007643. Appendix A of the user guide provides “experiment examples.” Also: Assay Types and Fluorophores QX Manager Software offers the assay methods explained in Table 19. Table 19. Assay types <table><tr><th>Assay type</th><th>Description</th></tr><tr><td>Single target per channel</td><td>Method assuming up to six probe colors (FAM/EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590) and up to six targets per channel. This is the default assay type, with the maximum six rows displayed. You can delete up to three rows.</td></tr><tr><td>Amplitude multiplex</td><td>Method to increase multiplexing up to twelve 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.</td></tr><tr><td>Probe mix triplex</td><td>Triplex mode that allows three 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. Group 3 has 1 target in ROX, 1 target in ATTO 590, and one target in both groups. You can delete a group, but not the individual targets within the group. Note: The channels within each group are not interchangeable therefore FAM is always paired with HEX/VIC and so forth.</td></tr><tr><td>Advanced classification</td><td>Method to increase multiplexing rate in any combination of targets detected per channel. This method requires manual classification of droplets.</td></tr><tr><td>Basic drop-off</td><td>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 each group are not interchangeable therefore FAM is always paired with HEX/VIC and Cy5 with Cy5.5.</td></tr></table>	Assay type	Description	Single target per channel	Method assuming up to six probe colors (FAM/EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590) and up to six targets per channel. This is the default assay type, with the maximum six rows displayed. You can delete up to three rows.	Amplitude multiplex	Method to increase multiplexing up to twelve 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 three 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. Group 3 has 1 target in ROX, 1 target in ATTO 590, and one target in both groups. You can delete a group, but not the individual targets within the group. Note: The channels within each 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 each group are not interchangeable therefore FAM is always paired with HEX/VIC and Cy5 with Cy5.5.
Assay type	Description												
Single target per channel	Method assuming up to six probe colors (FAM/EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590) and up to six targets per channel. This is the default assay type, with the maximum six rows displayed. You can delete up to three rows.												
Amplitude multiplex	Method to increase multiplexing up to twelve 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 three 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. Group 3 has 1 target in ROX, 1 target in ATTO 590, and one target in both groups. You can delete a group, but not the individual targets within the group. Note: The channels within each 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 each group are not interchangeable therefore FAM is always paired with HEX/VIC and Cy5 with Cy5.5.												

Claims

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Table 19. Assay types, continued

Assay type	Description
Double drop-off	This assay type is available only for the DOF experiment type, and allows three groups of three targets each. The first group appears by default, and looks for one target in FAM <u>and</u> in HEX/VIC (target type reference) one target in FAM (target type unknown), and one target in HEX/VIC (target type unknown). You can add the second group to look for one target in Cy5 <u>and</u> Cy5.5 (target type reference, with two reference copies), one target in Cy5 (target type unknown), and one target in Cy5.5 (target type unknown). You can also add a third group to look for one target in ROX <u>and</u> ATTO 590 (target type reference), one target in ROX (target type unknown), and one target in ATTO 590 (target type unknown). By default, all reference copies are set to 2. The channels are static for each group. (Channel 1 FAM is always paired with Channel 2 HEX/VIC, Channel 3 Cy5 is always paired with Channel 4 Cy5.5, and Channel 5 ROX is always paired with Channel 6 ATTO 590.

The experiment type you select determines the available assay methods. [Table 20](#) displays the sample type associated with each assay method.

Table 20. Assay types by experiment type

Assay type	Experiment type						
	DQ	CNV	MUT	DOF	GEX	RDQ	RED
Single target per channel	✓	✓	✓		✓	✓	✓
Amplitude multiplex	✓	✓	✓		✓	✓	✓
Probe mix triplex	✓	✓	✓		✓	✓	✓
Advanced classification	✓						✓
Basic drop-off				✓			
Double drop-off				✓			

Claims

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QX Manager Software automatically populates the fluorophore in each channel for each target but you can change the default selections.

Use [Table 21](#) to select from the fluorophore options for each assay method listed.

Table 21. Fluorophore options

Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4	Signal Ch5	Signal Ch6
Single target per channel, for 1 to 6 targets	FAM	HEX	Cy5	Cy5.5	ROX	ATTO590
	EvaGreen®	VIC	None	None	None	None
	None	None				
Amplitude multiplex, for 1 to 12 targets	FAM Hi	HEX Hi	Cy5 Hi	Cy5.5 Hi	ROX Hi	ATTO 590 Hi
	FAM Lo	HEX Lo	Cy 5 Lo	Cy 5.5 Lo	ROX Lo	ATTO 590 Lo
	EvaGreen® Hi	VIC Hi	None	None	None	None
	EvaGreen® Lo	VIC Lo				
	None	None				
Probe mix triplex, for 9 targets	FAM	HEX	Cy5	Cy5.5	ROX	ATTO 590
	None	VIC	None	None	None	None
		None				
Advanced classification	FAM	HEX	Cy5	Cy5.5	ROX	ATTO 590
	+1-10	1 to 10	1 to 10	1 to 10	1 to 10	1 to 10
	EvaGreen®	VIC	None	None	None	None
	1 to 10	1 to 10				
	None	None				

BIO_CC00007427 at 7505-7507; *see also* BIO_CC00007643.

Claims	Exemplary Disclosure of Accused Product																														
	Default color assignments for 2D amplitude plots are: <table><tr><td>Positive FAM</td><td>=</td><td>Blue</td></tr><tr><td>Positive HEX/VIC</td><td>=</td><td>Green</td></tr><tr><td>Positive FAM and HEX/VIC</td><td>=</td><td>Orange (may appear brown on some monitors)</td></tr><tr><td>Positive Cy5</td><td>=</td><td>Red</td></tr><tr><td>Positive Cy5.5</td><td>=</td><td>Violet</td></tr><tr><td>Postive Cy5 and Cy5.5</td><td>=</td><td>Light Blue</td></tr><tr><td>Positive ROX</td><td>=</td><td>Maroon</td></tr><tr><td>Positive ATTO 590</td><td>=</td><td>Gold</td></tr><tr><td>Positive ROX and ATTO 590</td><td>=</td><td>Aqua</td></tr><tr><td>Double negative</td><td>=</td><td>Gray</td></tr></table> BIO_CC00007427 at 7561; <i>see also</i> BIO_CC00007643.	Positive FAM	=	Blue	Positive HEX/VIC	=	Green	Positive FAM and HEX/VIC	=	Orange (may appear brown on some monitors)	Positive Cy5	=	Red	Positive Cy5.5	=	Violet	Postive Cy5 and Cy5.5	=	Light Blue	Positive ROX	=	Maroon	Positive ATTO 590	=	Gold	Positive ROX and ATTO 590	=	Aqua	Double negative	=	Gray
Positive FAM	=	Blue																													
Positive HEX/VIC	=	Green																													
Positive FAM and HEX/VIC	=	Orange (may appear brown on some monitors)																													
Positive Cy5	=	Red																													
Positive Cy5.5	=	Violet																													
Postive Cy5 and Cy5.5	=	Light Blue																													
Positive ROX	=	Maroon																													
Positive ATTO 590	=	Gold																													
Positive ROX and ATTO 590	=	Aqua																													
Double negative	=	Gray																													
[1a.3] wherein each hybridization probe comprises at least one fluorophore selected from four fluorophores.	The QX600 Droplet Digital PCR System is used to perform the step wherein each hybridization probe comprises at least one fluorophore selected from four fluorophores, both literally and under the doctrine of equivalents. For example:																														

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. Important: Use only the dyes listed above (FAM or EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590). Important: Your QX600 Droplet Reader performs the post-PCR droplet reading function only. A droplet generator and a thermal cycler are required to perform a full ddPCR cycle. You can purchase a droplet generator and thermal cycler, as well as associated accessories and consumables, from Bio-Rad. For information, see Appendix D, Ordering Information. BIO_CC00007427 at 7451; <i>see also</i> BIO_CC00007643.

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-glycosylase (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.

Claims	Exemplary Disclosure of Accused Product												
	Assay Types and Fluorophores QX Manager Software offers the assay methods explained in Table 19. Table 19. Assay types <table> <tr> <th>Assay type</th><th>Description</th></tr> <tr> <td>Single target per channel</td><td>Method assuming up to six probe colors (FAM/EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590) and up to six targets per channel. This is the default assay type, with the maximum six rows displayed. You can delete up to three rows.</td></tr> <tr> <td>Amplitude multiplex</td><td>Method to increase multiplexing up to twelve 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.</td></tr> <tr> <td>Probe mix triplex</td><td>Triplex mode that allows three 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. Group 3 has 1 target in ROX, 1 target in ATTO 590, and one target in both groups. You can delete a group, but not the individual targets within the group. Note: The channels within each group are not interchangeable therefore FAM is always paired with HEX/VIC and so forth.</td></tr> <tr> <td>Advanced classification</td><td>Method to increase multiplexing rate in any combination of targets detected per channel. This method requires manual classification of droplets.</td></tr> <tr> <td>Basic drop-off</td><td>This mode allows two groups of two targets each. The first group appears by default, with one target in FAM <u>and</u> HEX/VIC and one target in FAM <u>or</u> 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 <u>and</u> Cy5.5, one target in Cy5 <u>or</u> Cy5.5. You can delete a group, but not the individual targets in the group. Note: The channels within each group are not interchangeable therefore FAM is always paired with HEX/VIC and Cy5 with Cy5.5.</td></tr> </table>	Assay type	Description	Single target per channel	Method assuming up to six probe colors (FAM/EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590) and up to six targets per channel. This is the default assay type, with the maximum six rows displayed. You can delete up to three rows.	Amplitude multiplex	Method to increase multiplexing up to twelve 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 three 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. Group 3 has 1 target in ROX, 1 target in ATTO 590, and one target in both groups. You can delete a group, but not the individual targets within the group. Note: The channels within each 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 <u>and</u> HEX/VIC and one target in FAM <u>or</u> 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 <u>and</u> Cy5.5, one target in Cy5 <u>or</u> Cy5.5. You can delete a group, but not the individual targets in the group. Note: The channels within each group are not interchangeable therefore FAM is always paired with HEX/VIC and Cy5 with Cy5.5.
Assay type	Description												
Single target per channel	Method assuming up to six probe colors (FAM/EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590) and up to six targets per channel. This is the default assay type, with the maximum six rows displayed. You can delete up to three rows.												
Amplitude multiplex	Method to increase multiplexing up to twelve 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 three 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. Group 3 has 1 target in ROX, 1 target in ATTO 590, and one target in both groups. You can delete a group, but not the individual targets within the group. Note: The channels within each 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 <u>and</u> HEX/VIC and one target in FAM <u>or</u> 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 <u>and</u> Cy5.5, one target in Cy5 <u>or</u> Cy5.5. You can delete a group, but not the individual targets in the group. Note: The channels within each group are not interchangeable therefore FAM is always paired with HEX/VIC and Cy5 with Cy5.5.												

Table 19. Assay types, continued

Assay type	Description
Double drop-off	This assay type is available only for the DOF experiment type, and allows three groups of three targets each. The first group appears by default, and looks for one target in FAM <u>and</u> in HEX/VIC (target type reference) one target in FAM (target type unknown), and one target in HEX/VIC (target type unknown). You can add the second group to look for one target in Cy5 <u>and</u> Cy5.5 (target type reference, with two reference copies), one target in Cy5 (target type unknown), and one target in Cy5.5 (target type unknown). You can also add a third group to look for one target in ROX <u>and</u> ATTO 590 (target type reference), one target in ROX (target type unknown), and one target in ATTO 590 (target type unknown). By default, all reference copies are set to 2. The channels are static for each group. (Channel 1 FAM is always paired with Channel 2 HEX/VIC, Channel 3 Cy5 is always paired with Channel 4 Cy5.5, and Channel 5 ROX is always paired with Channel 6 ATTO 590.

The experiment type you select determines the available assay methods. [Table 20](#) displays the sample type associated with each assay method.

Table 20. Assay types by experiment type

Assay type	Experiment type						
	DQ	CNV	MUT	DOF	GEX	RDQ	RED
Single target per channel	✓	✓	✓		✓	✓	✓
Amplitude multiplex	✓	✓	✓		✓	✓	✓
Probe mix triplex	✓	✓	✓		✓	✓	✓
Advanced classification	✓						✓
Basic drop-off				✓			
Double drop-off				✓			

Claims

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QX Manager Software automatically populates the **fluorophore** in each channel for each target but you can change the default selections.

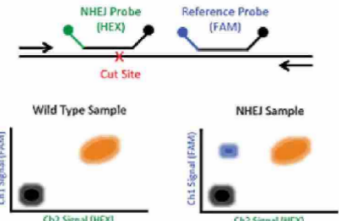
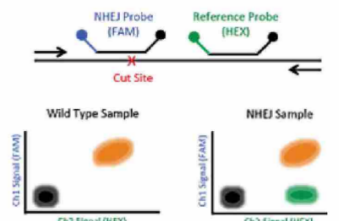
Use [Table 21](#) to select from the **fluorophore** options for each assay method listed.

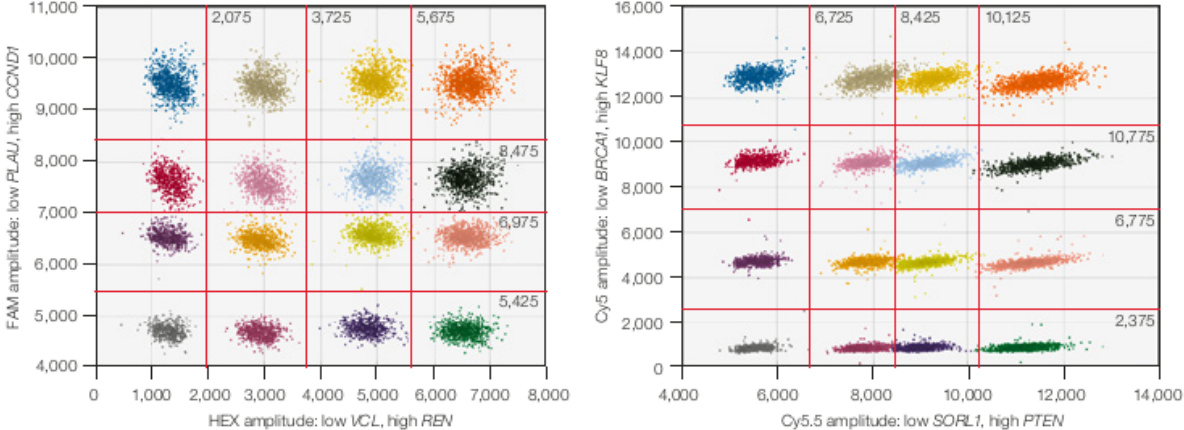
Table 21. Fluorophore options

Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4	Signal Ch5	Signal Ch6
Single target per channel, for 1 to 6 targets	FAM	HEX	Cy5	Cy5.5	ROX	ATTO590
	EvaGreen®	VIC	None	None	None	None
	None	None				
Amplitude multiplex, for 1 to 12 targets	FAM Hi	HEX Hi	Cy5 Hi	Cy5.5 Hi	ROX Hi	ATTO 590 Hi
	FAM Lo	HEX Lo	Cy 5 Lo	Cy 5.5 Lo	ROX Lo	ATTO 590 Lo
	EvaGreen® Hi	VIC Hi	None	None	None	None
	EvaGreen® Lo	VIC Lo				
	None	None				
Probe mix triplex, for 9 targets	FAM	HEX	Cy5	Cy5.5	ROX	ATTO 590
	None	VIC	None	None	None	None
Advanced classification	FAM	HEX	Cy5	Cy5.5	ROX	ATTO 590
	+1-10	1 to 10	1 to 10	1 to 10	1 to 10	1 to 10
	EvaGreen®	VIC	None	None	None	None
	1 to 10	1 to 10				
	None	None				

BIO_CC00007427 at 7504-7507; *see also* BIO_CC00007643.

The QX600 Droplet Digital PCR System uses assays and kits that use hybridization probes for the detection of targets. The following provides a non-limiting example disclosure for how the hybridization probes correspond to at least one fluorophore from the available fluorophore:

Claims	Exemplary Disclosure of Accused Product
	Drop-Off Probe in HEX NHEJ Probe = HEX and Reference Probe =FAM  Drop-Off Probe in FAM NHEJ Probe = FAM and Reference Probe =HEX  BIO_CCODE00007427 at 7618; <i>see also</i> BIO_CCODE00007643; <i>see</i> additional examples in Appendix A of the user guides. As shown in the example figure above, each probe corresponds to a specific fluorophore or a combination of fluorophores. The following provides an additional example of each probe comprising at least one fluorophore selected from a group of four fluorophores:

Claims	Exemplary Disclosure of Accused Product
	A  Left Plot: FAM amplitude: low PLAU, high CCND1 (Y-axis, 4,000 to 11,000) vs HEX amplitude: low VCL, high REV (X-axis, 0 to 8,000). Gated populations: 2,075, 3,725, 5,675, 8,475, 6,975, 5,425. Right Plot: Cy5 amplitude: low BRCA1, high KLF8 (Y-axis, 0 to 16,000) vs Cy5.5 amplitude: low SORL1, high PTEN (X-axis, 4,000 to 14,000). Gated populations: 6,725, 8,425, 10,125, 10,775, 6,775, 2,375. BIO_CC00008190 at 8192.
[1a.4] and wherein each hybridization probe, when excited and when contacted with its corresponding polynucleotide analyte, generates a cumulative intensity signal,	The QX600 Droplet Digital PCR System is used to perform the step wherein each hybridization probe, when excited and when contacted with its corresponding polynucleotide analyte, generates a cumulative intensity signal, both literally and under the doctrine of equivalents. The QX600 uses reagents and workflows similar to those used for most standard TaqMan probe-based assays:

Claims	Exemplary Disclosure of Accused Product
	Chapter 1 Introduction to Droplet Digital PCR Droplet Digital™ PCR (ddPCR™) is a digital polymerase chain reaction method based on water-oil emulsion droplet technology. ddPCR uses a combination of microfluidics and proprietary surfactant chemistries to divide each sample into water-in-oil droplets. The technology uses reagents and workflows similar to those used for most standard TaqMan probe-based assays, and provides absolute quantification of nucleic acid target sequences by counting nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions. ddPCR is highly effective in the following areas: ■ Absolute quantification — ddPCR provides a concentration of target DNA copies per input sample without the need for running standard curves, making this technique ideal for target DNA measurements, viral load analysis, and microbial quantification. ■ Genomic alterations such as gene copy number variation (CNV) — CNVs result in too few or too many dosage-sensitive genes responsible for phenotypic variability, complex behavioral traits, and disease. ddPCR enables measurement of 1.2x differences in gene copy number. ■ Detection of rare sequences — researchers must amplify single genes in a complex sample, such as a few tumor cells in a wild-type background. ddPCR is sensitive enough to detect rare mutations or sequences. ■ Gene expression and microRNA analysis — ddPCR provides stand-alone absolute quantification of expression levels, especially low-abundance microRNAs, with sensitivity and precision. ■ Next-generation sequencing (NGS) — ddPCR quantifies NGS sample library preparations to increase sequencing accuracy and reduce run repeats. Validate sequencing results such as single nucleotide polymorphisms or copy number variations with absolute quantification. ■ Single cell analysis — the high degree (10-fold to 100-fold) of cell-cell variation in gene expression and genomic content among homogeneous post-mitotic, progenitor, and stem cell populations drives a need for analysis from single cells. ddPCR enables low copy number quantification and gene expression of individual cells. ■ Genome edit detection — ddPCR enables fast, precise, and cost-effective assessment of HDR (Homology directed repair) and NHEJ (non-homologous end joining) generated by CRISPR-Cas9 or other genome editing tools. See BIO_CC00007427 at 7449; see also BIO_CC00007643. Under the process used by the QX600, the probe containing the fluorophores is mixed into the sample, e.g., during sample preparation/provision as described above. The sample then undergoes a thermal cycling as described above. Thereafter, the QX600 reader excites the fluorophores on the probes in the sample using, for example and on information and belief, light at different wavelengths. This results in the hybridization probe (and the fluorophore component thereof) becoming excited. If the

Claims	Exemplary Disclosure of Accused Product
	polynucleotide analyte is in contact with the hybridization probe, a cumulative intensity signal is generated. An exemplary process for sample preparation wherein the hybridization probe is excited placed into contact with its corresponding polynucleotide analyte is describe at https://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6407.pdf at 4-5, 12-13, 15-17. On information and belief, the same or materially similar process is followed for preparing samples for the QX600. See <i>e.g.</i>, BIO_CC00007875, BIO_CC00007877, BIO_CC00007879, and BIO_CC00007881. The result is that the sample exhibits varying degrees of dye fluorescence which represent the cumulative intensity signals. 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.

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. Important: Use only the dyes listed above (FAM or EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590). Important: Your QX600 Droplet Reader performs the post-PCR droplet reading function only. A droplet generator and a thermal cycler are required to perform a full ddPCR cycle. You can purchase a droplet generator and thermal cycler, as well as associated accessories and consumables, from Bio-Rad. For information, see Appendix D, Ordering Information. BIO_CC00007427 at 7450-7451; <i>see also</i> BIO_CC00007643.

Claims

Exemplary Disclosure of Accused Product

QX Manager Software automatically populates the fluorophore in each channel for each target but you can change the default selections.

Use [Table 21](#) to select from the fluorophore options for each assay method listed.

Table 21. Fluorophore options

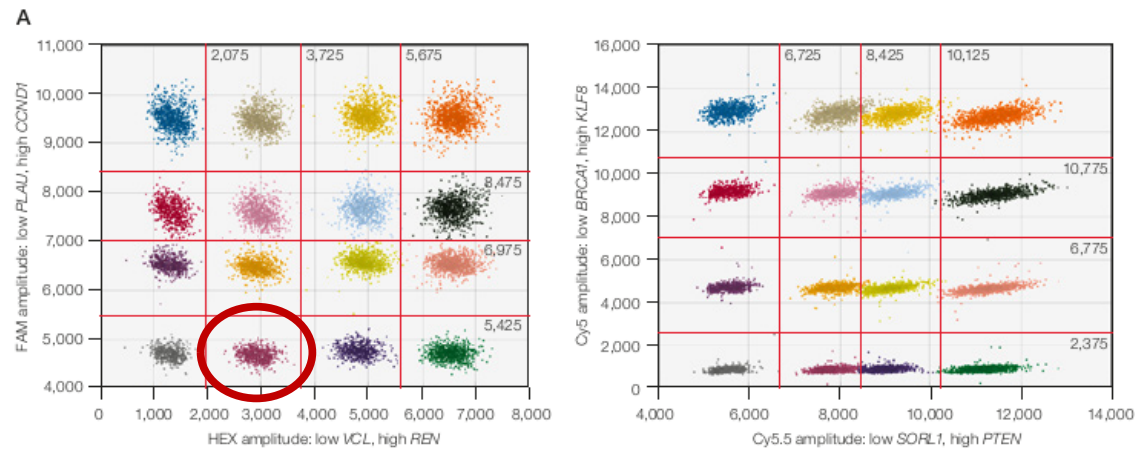
Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4	Signal Ch5	Signal Ch6
Single target per channel, for 1 to 8 targets	FAM	HEX	Cy5	Cy5.5	ROX	ATTO590
	EvaGreen®	VIC	None	None	None	None
	None	None				
Amplitude multiplex, for 1 to 12 targets	FAM Hi	HEX Hi	Cy5 Hi	Cy5.5 Hi	ROX Hi	ATTO 590 Hi
	FAM Lo	HEX Lo	Cy5 Lo	Cy5.5 Lo	ROX Lo	ATTO 590 Lo
	EvaGreen® Hi	VIC Hi	None	None	None	None
	EvaGreen® Lo	VIC Lo				
	None	None				
Probe mix triplex, for 9 targets	FAM	HEX	Cy5	Cy5.5	ROX	ATTO 590
	None	VIC	None	None	None	None
		None				
Advanced classification	FAM	HEX	Cy5	Cy5.5	ROX	ATTO 590
	+1-10	1 to 10	1 to 10	1 to 10	1 to 10	1 to 10
	EvaGreen®	VIC	None	None	None	None
	1 to 10	1 to 10				
	None	None				

BIO_CCODE00007427 at 7507; *see also* BIO_CCODE00007643.

Claims	Exemplary Disclosure of Accused Product
	Analysis Windows In its Analysis module, QX Manager Software provides amplitude displays and data visualization charts in the Analysis module. For more information on each chart, see Chapter 8, Data Analysis Methodology. Amplitude Charts Amplitude charts illustrate the droplets by degree of dye fluorescence. QX Manager Software displays fluorescence for positive and negative targets identified with FAM/EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590 fluorophores in the following analysis windows: ■ 1D Amplitude ■ 2D Amplitude ■ 3D Amplitude Data Visualization Charts Data visualization charts show calculations based on positive droplet concentrations. QX Manager Software counts the number of positive and negative droplets for each fluorophore in each sample, and then fits the fraction of positive droplets to a Poisson algorithm to determine the starting concentration of the target DNA molecule in units of copies/μL input in the following analysis windows: ■ Concentration ■ Copy Number ■ Ratio ■ Fractional Abundance When you open the analysis data file in QX Manager Software and select one or more wells in the grid, the software plots each calculated Poisson distribution average as a data point in the grid. You can pause on the data point to see the high, median, and low numbers. Note: You can display the data using a confidence level of either 68% or 95% (default setting). Event Counts Event counts record the number of droplets in which the target was found (positive) and was not found (negative). The Event Counts tab shows a bar chart view of the number of positive, negative, and total droplets for each of the targets in each of the selected wells. BIO_CC00007427 at 7535; <i>see also</i> BIO_CC00007643. The following exemplary image pictorially depicts some cumulative intensity signals:

Claims

Exemplary Disclosure of Accused Product



BIO_CCODE00008190 at BIO_CCODE00008192; see discussion in Step 1a.3 above.

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. <table><tr><th>ddPCR Supermix</th><th>Description</th></tr><tr><td>Multiplex Supermix</td><td>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.</td></tr><tr><td>Supermix for Probes (No dUTP)</td><td>For use in nucleic acid sample preparations, with sensitivity for the amplification and detection of DNA targets using hydrolysis probe-based assays.</td></tr><tr><td>Supermix for Probes</td><td>For use in sample preparation with uracil N-glucosylase (UNG) decontamination protocols to prevent the reamplification of carryover PCR products between experiments.</td></tr></table> BIO_CC00007427 at 7504. The QX600 system performs and describes the general functionality of hybridization probes used in amplification reaction detections, including in amplification and detection of DNA targets using hydrolysis probe-based assays as described for the Supermix for Probes (No dUTP). The 2D amplitude plots show multiple droplet clusters which individually correspond to a cumulative intensity signal generated when each probe is excited and contacted with its corresponding polynucleotide analyte targets. For example, the bottom left magenta cluster in the left graph (circled in magenta) corresponds to the VCL target probe excited and contacted with its corresponding VCL target to generate a cumulative intensity signal shown as a magenta cluster on the 2D amplitude plot. Thus, when each hybridization probe (e.g., VCL probe) is excited via hydrolysis probe-based assays, and contacted with its corresponding polynucleotide analyte (e.g., VCL), it generates a cumulative intensity signal (e.g., the magenta clusters in 2D amplitude plots).	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.
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.								

Claims	Exemplary Disclosure of Accused Product
[1a.5] further wherein each generated cumulative intensity signal comprises at least one signal generated by excitement of at least one of the four fluorophores;	Plaintiffs incorporate their discussion from [1a.4] above here. When the hybridization probes are excited in accordance with performing step 1a.4 above, the fluorophore becomes amplified/excited by the same process. See https://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6407.pdf at 4-5, 12-13. On information and belief, the same or materially similar process is followed for preparing samples for the QX600. See also: 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.

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. Important: Use only the dyes listed above (FAM or EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590). Important: Your QX600 Droplet Reader performs the post-PCR droplet reading function only. A droplet generator and a thermal cycler are required to perform a full ddPCR cycle. You can purchase a droplet generator and thermal cycler, as well as associated accessories and consumables, from Bio-Rad. For information, see Appendix D, Ordering Information. BIO_CC00007427 at 7450-7451; <i>see also</i> BIO_CC00007643.

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. <table><thead><tr><th>ddPCR Supermix</th><th>Description</th></tr></thead><tbody><tr><td>Multiplex Supermix</td><td>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.</td></tr><tr><td>Supermix for Probes (No dUTP)</td><td>For use in nucleic acid sample preparations, with sensitivity for the amplification and detection of DNA targets using hydrolysis probe-based assays.</td></tr><tr><td>Supermix for Probes</td><td>For use in sample preparation with uracil N-glucosylase (UNG) decontamination protocols to prevent the reamplification of carryover PCR products between experiments.</td></tr><tr><td>Supermix for Residual DNA Quantification</td><td>For use in residual DNA detection.</td></tr><tr><td>One-Step RT Advanced Kit for Probe</td><td>For use in absolute quantification of target RNA molecules.</td></tr><tr><td>EvaGreen® Supermix</td><td>For use in gene expression.</td></tr></tbody></table> BIO_CC00007427 at 7504; <i>see also</i> BIO_CC00007643.	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.
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.														

Claims

Exemplary Disclosure of Accused Product

QX Manager Software automatically populates the fluorophore in each channel for each target but you can change the default selections.

Use [Table 21](#) to select from the fluorophore options for each assay method listed.

Table 21. Fluorophore options

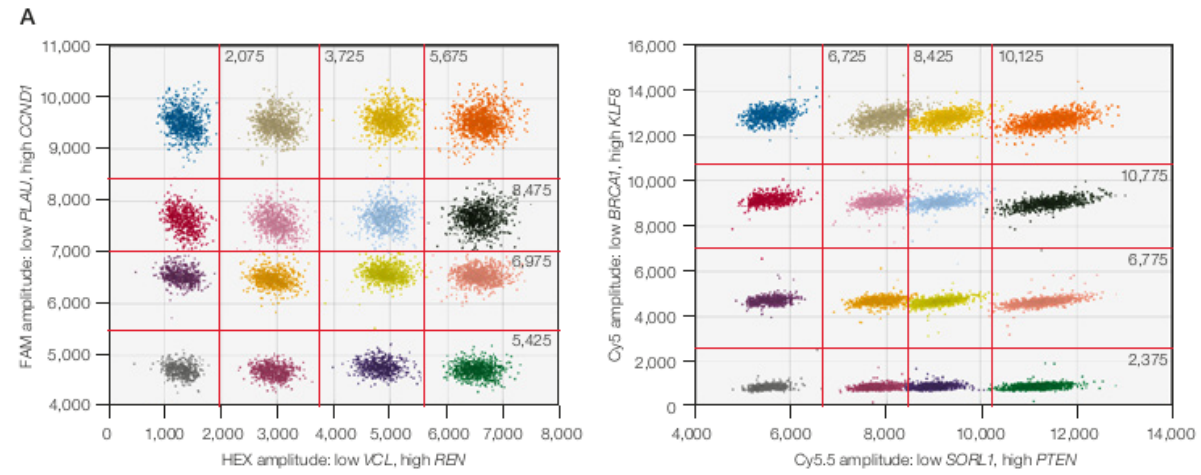
Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4	Signal Ch5	Signal Ch6
Single target per channel, for 1 to 8 targets	FAM	HEX	Cy5	Cy5.5	ROX	ATTIO590
	EvaGreen®	VIC	None	None	None	None
	None	None				
Amplitude multiplex, for 1 to 12 targets	FAM Hi	HEX Hi	Cy5 Hi	Cy5.5 Hi	ROX Hi	ATTO 590 Hi
	FAM Lo	HEX Lo	Cy5 Lo	Cy5.5 Lo	ROX Lo	ATTO 590 Lo
	EvaGreen® Hi	VIC Hi	None	None	None	None
	EvaGreen® Lo	VIC Lo				
	None	None				
Probe mix triplex, for 9 targets	FAM	HEX	Cy5	Cy5.5	ROX	ATTO 590
	None	VIC	None	None	None	None
		None				
Advanced classification	FAM	HEX	Cy5	Cy5.5	ROX	ATTO 590
	+1-10	1 to 10	1 to 10	1 to 10	1 to 10	1 to 10
	EvaGreen®	VIC	None	None	None	None
	1 to 10	1 to 10				
	None	None				

BIO_CCODE00007427 at 7507; *see also* BIO_CCODE00007643.

Claims	Exemplary Disclosure of Accused Product
	Analysis Windows In its Analysis module, QX Manager Software provides amplitude displays and data visualization charts in the Analysis module. For more information on each chart, see Chapter 8, Data Analysis Methodology. Amplitude Charts Amplitude charts illustrate the droplets by degree of dye fluorescence. QX Manager Software displays fluorescence for positive and negative targets identified with FAM/EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590 fluorophores in the following analysis windows: ■ 1D Amplitude ■ 2D Amplitude ■ 3D Amplitude Data Visualization Charts Data visualization charts show calculations based on positive droplet concentrations. QX Manager Software counts the number of positive and negative droplets for each fluorophore in each sample, and then fits the fraction of positive droplets to a Poisson algorithm to determine the starting concentration of the target DNA molecule in units of copies/μL input in the following analysis windows: ■ Concentration ■ Copy Number ■ Ratio ■ Fractional Abundance When you open the analysis data file in QX Manager Software and select one or more wells in the grid, the software plots each calculated Poisson distribution average as a data point in the grid. You can pause on the data point to see the high, median, and low numbers. Note: You can display the data using a confidence level of either 68% or 95% (default setting). Event Counts Event counts record the number of droplets in which the target was found (positive) and was not found (negative). The Event Counts tab shows a bar chart view of the number of positive, negative, and total droplets for each of the targets in each of the selected wells. BIO_CC00007427 at 7535; <i>see also</i> BIO_CC00007643. The following exemplary image pictorially depicts some cumulative intensity signals:

Claims

Exemplary Disclosure of Accused Product



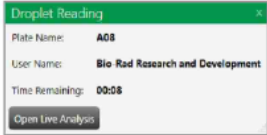
See discussion in Step 1a.3 above.

Claims	Exemplary Disclosure of Accused Product
[1b] b) contacting said sample solution volume with the plurality of hybridization probes and exciting the hybridization probes to generate the cumulative intensity signal(s) if one or more of the at least seven polynucleotide analytes is present in the sample volume; and	Plaintiffs incorporate by reference the discussion from Steps 1a.4 and 1a.5. When the process described above is performed using a QX600, the sample solution described above in step 1a will be contacted with the various hybridization probes and excited through the process described in Steps 1a.4 and 1a.5. The cumulative intensity signals are generated in accordance with Step 1a.4 and 1a.5 based on the hybridization probes contacting the corresponding polynucleotide. The “at least seven” limitation occurs when contact is made between the hybridization probes and at least seven corresponding polynucleotides in accordance with the description in Step 1a.
[1c.1] c) measuring the cumulative intensity signal(s)	The QX600 Droplet Digital PCR System is used to perform the step measuring the cumulative intensity signal(s), both literally and under the doctrine of equivalents. The QX600 Reader is used to measure the cumulative intensity signals.

Claims	Exemplary Disclosure of Accused Product
	Chapter 1 Introduction to Droplet Digital PCR Droplet Digital™ PCR (ddPCR™) is a digital polymerase chain reaction method based on water-oil emulsion droplet technology. ddPCR uses a combination of microfluidics and proprietary surfactant chemistries to divide each sample into water-in-oil droplets. The technology uses reagents and workflows similar to those used for most standard TaqMan probe-based assays, and provides absolute quantification of nucleic acid target sequences by counting nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions. ddPCR is highly effective in the following areas: ■ Absolute quantification — ddPCR provides a concentration of target DNA copies per input sample without the need for running standard curves, making this technique ideal for target DNA measurements, viral load analysis, and microbial quantification. ■ Genomic alterations such as gene copy number variation (CNV) — CNVs result in too few or too many dosage-sensitive genes responsible for phenotypic variability, complex behavioral traits, and disease. ddPCR enables measurement of 1.2x differences in gene copy number. ■ Detection of rare sequences — researchers must amplify single genes in a complex sample, such as a few tumor cells in a wild-type background. ddPCR is sensitive enough to detect rare mutations or sequences. ■ Gene expression and microRNA analysis — ddPCR provides stand-alone absolute quantification of expression levels, especially low-abundance microRNAs, with sensitivity and precision. ■ Next-generation sequencing (NGS) — ddPCR quantifies NGS sample library preparations to increase sequencing accuracy and reduce run repeats. Validate sequencing results such as single nucleotide polymorphisms or copy number variations with absolute quantification. ■ Single cell analysis — the high degree (10-fold to 100-fold) of cell-cell variation in gene expression and genomic content among homogeneous post-mitotic, progenitor, and stem cell populations drives a need for analysis from single cells. ddPCR enables low copy number quantification and gene expression of individual cells. ■ Genome edit detection — ddPCR enables fast, precise, and cost-effective assessment of HDR (Homology directed repair) and NHEJ (non-homologous end joining) generated by CRISPR-Cas9 or other genome editing tools.

Claims	Exemplary Disclosure of Accused Product
	ddPCR has the following benefits for nucleic acid quantification: ■ Unparalleled precision — The massive sample partitioning afforded by ddPCR enables small fold differences in target DNA sequence between samples to be reliably measured. ■ Increased signal-to-noise ratio — High-copy templates and background are diluted, effectively enriching template concentration in target-positive partitions. This allows for the sensitive detection of rare targets and enables a $\pm 10\%$ precision in quantification. ■ Removal of PCR efficiency bias — Error rates are reduced by removing the amplification efficiency reliance of qPCR, enabling accurate quantification of targets to near zero. ■ Simplified quantification — There is no requirement for a standard curve for absolute quantification. 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. Finding Out More Click the Help tab (?) on the left, and then click the Bio-Rad Website to access links to technical notes, manuals, videos, product information, and technical support. The website also provides many technical resources on a wide variety of methods and applications related to PCR, Droplet Digital PCR, and gene expression. See BIO_CC00007427 at 7449-7450; see also BIO_CC00007643.

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. Important: Use only the dyes listed above (FAM or EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590). Important: Your QX600 Droplet Reader performs the post-PCR droplet reading function only. A droplet generator and a thermal cycler are required to perform a full ddPCR cycle. You can purchase a droplet generator and thermal cycler, as well as associated accessories and consumables, from Bio-Rad. For information, see Appendix D, Ordering Information. <i>See BIO_CC00007427 at 7451; see also BIO_CC00007643.</i>

Claims	Exemplary Disclosure of Accused Product
	Using Live Analysis When the QX600 Droplet Reader initiates droplet reading, QX Manager Software provides an option for real-time analysis of the wells that have been read, while the process as a whole is still active. QX Manager Software initially shows each well in the Plate Editor as disabled. As the software confirms that the instrument has acquired a well and read the droplets, the software enables the well for live analysis in read-only mode. The process continues until all wells are enabled and the process is finished. To use live analysis 1. Tap the droplet reading icon (📊) for the run in progress. The Droplet Reading dialog box opens.  2. Click Open Live Analysis. 3. Select an analysis window. 4. In the well selector, select one or more wells that are enabled. Important: QX Manager Software displays read-only data in the selected analysis window. You can view the data but to make changes, you must wait for the run to complete, and then change the data in the Analysis module. <i>See BIO_CC00007427 at 7492; see also BIO_CC00007643.</i>

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. <table><tr><th>ddPCR Supermix</th><th>Description</th></tr><tr><td>Multiplex Supermix</td><td>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.</td></tr><tr><td>Supermix for Probes (No dUTP)</td><td>For use in nucleic acid sample preparations, with sensitivity for the amplification and detection of DNA targets using hydrolysis probe-based assays.</td></tr><tr><td>Supermix for Probes</td><td>For use in sample preparation with uracil N-glucosylase (UNG) decontamination protocols to prevent the reamplification of carryover PCR products between experiments.</td></tr></table> BIO_CC00007427 at 7504; <i>see also</i> BIO_CC00007643. The QX600 system provides ways of measuring the cumulative intensity signals, for example, signal from the hydrolysis probes is generated, for example, when they become attached to the analytes targets and is generated by means of an extension reaction by common hydrolysis probe chemistry in using hydrolysis probe-based assays.	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.
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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.								

Claims		Exemplary Disclosure of Accused Product									
		<table><tr><th colspan="2">Table 18. Experiment types</th></tr><tr><th>Type</th><th>Description</th></tr><tr><td>Direct quantification (DQ)</td><td>DQ uses absolute quantification to determine the concentration (copies/μl) of target DNA copies in a sample. DQ measures the number of positive and negative droplets for each target in a well based on their fluorescence amplitude, and uses a Poisson algorithm to calculate the starting concentration of each target DNA molecule. Note: The ABS experiment type created in QuantaSoft Software versions 1.4 through 1.7 is automatically mapped into the software as a DQ experiment type.</td></tr><tr><td>Copy number variation (CNV)</td><td>CNV determines concentration and calculates the copy number of an unknown (CNV) target relative to a known reference or references within the same well.</td></tr></table>	Table 18. Experiment types		Type	Description	Direct quantification (DQ)	DQ uses absolute quantification to determine the concentration (copies/μl) of target DNA copies in a sample. DQ measures the number of positive and negative droplets for each target in a well based on their fluorescence amplitude, and uses a Poisson algorithm to calculate the starting concentration of each target DNA molecule. Note: The ABS experiment type created in QuantaSoft Software versions 1.4 through 1.7 is automatically mapped into the software as a DQ experiment type.	Copy number variation (CNV)	CNV determines concentration and calculates the copy number of an unknown (CNV) target relative to a known reference or references within the same well.	
Table 18. Experiment types											
Type	Description										
Direct quantification (DQ)	DQ uses absolute quantification to determine the concentration (copies/μl) of target DNA copies in a sample. DQ measures the number of positive and negative droplets for each target in a well based on their fluorescence amplitude, and uses a Poisson algorithm to calculate the starting concentration of each target DNA molecule. Note: The ABS experiment type created in QuantaSoft Software versions 1.4 through 1.7 is automatically mapped into the software as a DQ experiment type.										
Copy number variation (CNV)	CNV determines concentration and calculates the copy number of an unknown (CNV) target relative to a known reference or references within the same well.										
	BIO_CCODE00007427 at 7501; <i>see also</i> BIO_CCODE00007643.										

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	Assay Types and Fluorophores QX Manager Software offers the assay methods explained in Table 19. Table 19. Assay types <table><tr><th>Assay type</th><th>Description</th></tr><tr><td>Single target per channel</td><td>Method assuming up to six probe colors (FAM/EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590) and up to six targets per channel. This is the default assay type, with the maximum six rows displayed. You can delete up to three rows.</td></tr><tr><td>Amplitude multiplex</td><td>Method to increase multiplexing up to twelve 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.</td></tr><tr><td>Probe mix triplex</td><td>Triplex mode that allows three 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. Group 3 has 1 target in ROX, 1 target in ATTO 590, and one target in both groups. You can delete a group, but not the individual targets within the group. Note: The channels within each group are not interchangeable therefore FAM is always paired with HEX/VIC and so forth.</td></tr><tr><td>Advanced classification</td><td>Method to increase multiplexing rate in any combination of targets detected per channel. This method requires manual classification of droplets.</td></tr><tr><td>Basic drop-off</td><td>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 each group are not interchangeable therefore FAM is always paired with HEX/VIC and Cy5 with Cy5.5.</td></tr></table>	Assay type	Description	Single target per channel	Method assuming up to six probe colors (FAM/EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590) and up to six targets per channel. This is the default assay type, with the maximum six rows displayed. You can delete up to three rows.	Amplitude multiplex	Method to increase multiplexing up to twelve 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 three 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. Group 3 has 1 target in ROX, 1 target in ATTO 590, and one target in both groups. You can delete a group, but not the individual targets within the group. Note: The channels within each 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 each group are not interchangeable therefore FAM is always paired with HEX/VIC and Cy5 with Cy5.5.
Assay type	Description												
Single target per channel	Method assuming up to six probe colors (FAM/EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590) and up to six targets per channel. This is the default assay type, with the maximum six rows displayed. You can delete up to three rows.												
Amplitude multiplex	Method to increase multiplexing up to twelve 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 three 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. Group 3 has 1 target in ROX, 1 target in ATTO 590, and one target in both groups. You can delete a group, but not the individual targets within the group. Note: The channels within each 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 each group are not interchangeable therefore FAM is always paired with HEX/VIC and Cy5 with Cy5.5.												

Table 19. Assay types, continued

Assay type	Description
Double drop-off	This assay type is available only for the DOF experiment type, and allows three groups of three targets each. The first group appears by default, and looks for one target in FAM <u>and</u> in HEX/VIC (target type reference) one target in FAM (target type unknown), and one target in HEX/VIC (target type unknown). You can add the second group to look for one target in Cy5 <u>and</u> Cy5.5 (target type reference, with two reference copies), one target in Cy5 (target type unknown), and one target in Cy5.5 (target type unknown). You can also add a third group to look for one target in ROX <u>and</u> ATTO 590 (target type reference), one target in ROX (target type unknown), and one target in ATTO 590 (target type unknown). By default, all reference copies are set to 2. The channels are static for each group. (Channel 1 FAM is always paired with Channel 2 HEX/VIC, Channel 3 Cy5 is always paired with Channel 4 Cy5.5, and Channel 5 ROX is always paired with Channel 6 ATTO 590.

The experiment type you select determines the available assay methods. [Table 20](#) displays the sample type associated with each assay method.

Table 20. Assay types by experiment type

Assay type	Experiment type						
	DQ	CNV	MUT	DOF	GEX	RDQ	RED
Single target per channel	✓	✓	✓		✓	✓	✓
Amplitude multiplex	✓	✓	✓		✓	✓	✓
Probe mix triplex	✓	✓	✓		✓	✓	✓
Advanced classification	✓						✓
Basic drop-off				✓			
Double drop-off				✓			

Claims	Exemplary Disclosure of Accused Product																																																																																																												
	QX Manager Software automatically populates the fluorophore in each channel for each target but you can change the default selections. Use Table 21 to select from the fluorophore options for each assay method listed. Table 21. Fluorophore options <table><tr><th>Assay method</th><th>Signal Ch1</th><th>Signal Ch2</th><th>Signal Ch3</th><th>Signal Ch4</th><th>Signal Ch5</th><th>Signal Ch6</th></tr><tr><td rowspan="3">Single target per channel, for 1 to 6 targets</td><td>FAM</td><td>HEX</td><td>Cy5</td><td>Cy5.5</td><td>ROX</td><td>ATTIO590</td></tr><tr><td>EvaGreen®</td><td>VIC</td><td>None</td><td>None</td><td>None</td><td>None</td></tr><tr><td>None</td><td>None</td><td></td><td></td><td></td><td></td></tr><tr><td rowspan="5">Amplitude multiplex, for 1 to 12 targets</td><td>FAM Hi</td><td>HEX Hi</td><td>Cy5 Hi</td><td>Cy5.5 Hi</td><td>ROX Hi</td><td>ATTO 590 Hi</td></tr><tr><td>FAM Lo</td><td>HEX Lo</td><td>Cy 5 Lo</td><td>Cy 5.5 Lo</td><td>ROX Lo</td><td>ATTO 590 Lo</td></tr><tr><td>EvaGreen® Hi</td><td>VIC Hi</td><td>None</td><td>None</td><td>None</td><td>None</td></tr><tr><td>EvaGreen® Lo</td><td>VIC Lo</td><td></td><td></td><td></td><td></td></tr><tr><td>None</td><td>None</td><td></td><td></td><td></td><td></td></tr><tr><td rowspan="3">Probe mix triplex, for 9 targets</td><td>FAM</td><td>HEX</td><td>Cy5</td><td>Cy5.5</td><td>ROX</td><td>ATTO 590</td></tr><tr><td>None</td><td>VIC</td><td>None</td><td>None</td><td>None</td><td>None</td></tr><tr><td></td><td>None</td><td></td><td></td><td></td><td></td></tr><tr><td rowspan="4">Advanced classification</td><td>FAM</td><td>HEX</td><td>Cy5</td><td>Cy5.5</td><td>ROX</td><td>ATTO 590</td></tr><tr><td>+1-10</td><td>1 to 10</td><td>1 to 10</td><td>1 to 10</td><td>1 to 10</td><td>1 to 10</td></tr><tr><td>EvaGreen®</td><td>VIC</td><td>None</td><td>None</td><td>None</td><td>None</td></tr><tr><td>1 to 10</td><td>1 to 10</td><td></td><td></td><td></td><td></td></tr><tr><td></td><td>None</td><td>None</td><td></td><td></td><td></td><td></td></tr></table> BIO_CC00007427 at 7505-7507; <i>see also</i> BIO_CC00007643. The QX600 system is used to perform different experiment and assay types to measure the cumulative intensity signals, such as amplitude multiplex and probe mix triplex experiments and assays.	Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4	Signal Ch5	Signal Ch6	Single target per channel, for 1 to 6 targets	FAM	HEX	Cy5	Cy5.5	ROX	ATTIO590	EvaGreen®	VIC	None	None	None	None	None	None					Amplitude multiplex, for 1 to 12 targets	FAM Hi	HEX Hi	Cy5 Hi	Cy5.5 Hi	ROX Hi	ATTO 590 Hi	FAM Lo	HEX Lo	Cy 5 Lo	Cy 5.5 Lo	ROX Lo	ATTO 590 Lo	EvaGreen® Hi	VIC Hi	None	None	None	None	EvaGreen® Lo	VIC Lo					None	None					Probe mix triplex, for 9 targets	FAM	HEX	Cy5	Cy5.5	ROX	ATTO 590	None	VIC	None	None	None	None		None					Advanced classification	FAM	HEX	Cy5	Cy5.5	ROX	ATTO 590	+1-10	1 to 10	1 to 10	1 to 10	1 to 10	1 to 10	EvaGreen®	VIC	None	None	None	None	1 to 10	1 to 10						None	None				
Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4	Signal Ch5	Signal Ch6																																																																																																							
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	EvaGreen®	VIC	None	None	None	None																																																																																																							
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Amplitude multiplex, for 1 to 12 targets	FAM Hi	HEX Hi	Cy5 Hi	Cy5.5 Hi	ROX Hi	ATTO 590 Hi																																																																																																							
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	EvaGreen® Hi	VIC Hi	None	None	None	None																																																																																																							
	EvaGreen® Lo	VIC Lo																																																																																																											
	None	None																																																																																																											
Probe mix triplex, for 9 targets	FAM	HEX	Cy5	Cy5.5	ROX	ATTO 590																																																																																																							
	None	VIC	None	None	None	None																																																																																																							
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	1 to 10	1 to 10																																																																																																											
	None	None																																																																																																											
[1c.2] thereby generating a cumulative signal measurement.	The QX600 Droplet Digital PCR System is used to perform the step generating a cumulative signal measurement, wherein the cumulative signal measurement non-degenerately indicates the presence or absence of the at least seven polynucleotide analytes, in any combination of presence or absence, both literally and under the doctrine of equivalents.																																																																																																												

Claims	Exemplary Disclosure of Accused Product
wherein the cumulative signal measurement non-degenerately indicates the presence or absence of the at least seven polynucleotide analytes, in any combination of presence or absence,	For example: Setting Up Experiment Parameters To set experiment parameters 1. Open the analysis data file and select the Plate Editor tab. 2. Select one or more wells to be assigned well analysis attributes. 3. Change the experiment type to Direct Quantification (DQ), and then click Apply. 4. (Optional) Change the sample description identifiers. 5. (Optional) Change the sample type. The default type is Unknown. Tip: For automatic thresholding of positive control wells, you must select Pos Ctrl. 6. Under Assay Type, select Amplitude Multiplex and click Apply. Twelve target rows appear. 7. Change the target name and type (unknown or reference) for each target in the well. The software defaults to FAM Lo, FAM Hi, HEX Lo, HEX Hi, Cy5 Lo, Cy5 Hi, Cy5.5 Lo, Cy5.5 Hi, ROX Lo, ROX Hi, ATTO 590 Lo, and ATTO 590 Hi, respectively, for each target. 8. (Optional) Change the high and low target identifiers. Amplitude Multiplex allows for up to two assays in each channel, indicated by Lo and Hi labels. In this multiplex mode, each target may have a signal for only one channel. You can ■ Select a different dye, if the channel supports two dyes (for example change HEX to VIC or VIC to HEX) ■ Select a different channel for the target (for example, change FAM to HEX) ■ Change Hi to Lo or Lo to Hi 9. Click Apply. BIO CCODE00007427 at 7612; <i>see also</i> BIO_CCODE00007643. The QX600 System defaults to FAM Lo, FAM Hi, HEX Lo, HEX Hi, Cy5 Lo, Cy5 Hi, Cy5.5 Lo, Cy5.5 Hi, ROX Lo, ROX Hi, ATTO 590 Lo, and ATTO 590 Hi, respectively, for each target. Thus, on information and belief, each target is encoded with a specific fluorophore corresponding to a cumulative signal measurement to non-degenerately indicate the presence or absence of each target.

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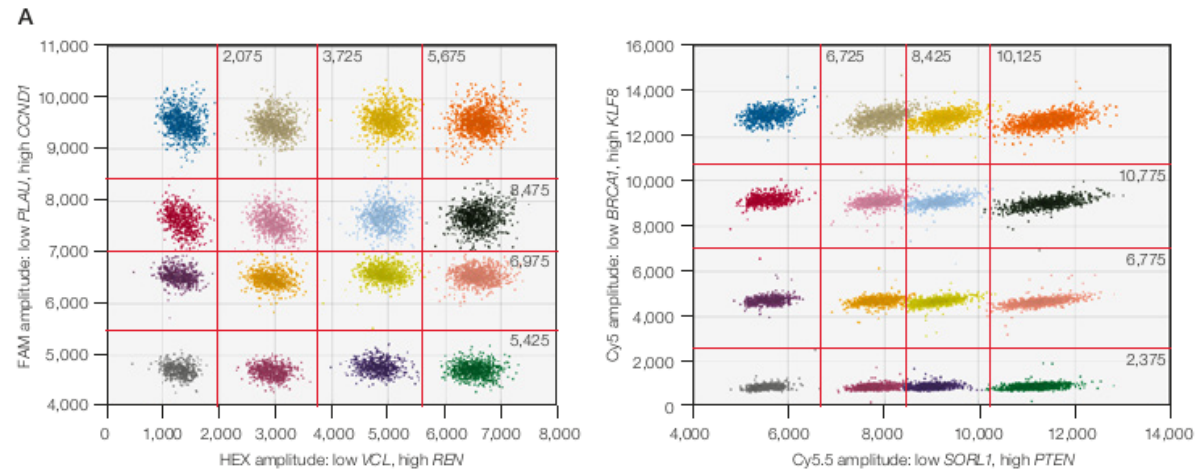
QX Manager Software automatically populates the fluorophore in each channel for each target but you can change the default selections.

Use [Table 21](#) to select from the fluorophore options for each assay method listed.

Table 21. Fluorophore options

Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4	Signal Ch5	Signal Ch6
Single target per channel, for 1 to 6 targets	FAM	HEX	Cy5	Cy5.5	ROX	ATTO590
	EvaGreen®	VIC	None	None	None	None
	None	None				
Amplitude multiplex, for 1 to 12 targets	FAM Hi	HEX Hi	Cy5 Hi	Cy5.5 Hi	ROX Hi	ATTO 590 Hi
	FAM Lo	HEX Lo	Cy 5 Lo	Cy 5.5 Lo	ROX Lo	ATTO 590 Lo
	EvaGreen® Hi	VIC Hi	None	None	None	None
	EvaGreen® Lo	VIC Lo				
	None	None				
Probe mix triplex, for 9 targets	FAM	HEX	Cy5	Cy5.5	ROX	ATTO 590
	None	VIC	None	None	None	None
Advanced classification	FAM	HEX	Cy5	Cy5.5	ROX	ATTO 590
	+1-10	1 to 10	1 to 10	1 to 10	1 to 10	1 to 10
	EvaGreen®	VIC	None	None	None	None
	1 to 10	1 to 10				
	None	None				

BIO CCODE00007427 at 7507; *see also* BIO_CCODE00007643.



BIO_CCODE00008190 at 8192.

As seen in the exemplary plots above, when the QX600 is used, each cumulative signal measurement gives a unique pattern that, on information and belief, non-degenerately indicates the presence or absence of the at least seven polynucleotide analytes (*e.g.*, eight targets), in any combination of presence or absence. On information and belief, this may be based on the specific encoding scheme chosen.

Also, on information and belief, the specific exemplary plots above were selected to non-degenerately indicate the presence or absence of eight targets in any combination presence or absence. The x and y axes of the two 2D amplitude plots indicate the targets and their corresponding fluorophore components (*e.g.*, “HEX amplitude: low VCL, high REN” indicates VCL target corresponds to low HEX signal, REN target corresponds to high HEX signal). Low is represented by 1 (*e.g.*, low HEX for VCL indicated as 1), and high is represented by 2 (*e.g.*, high HEX for REN indicated as 2). The assay as reproduced above shows the non-degenerate identification of 8 analytes across four channels. Each graph represents 2^4 possibilities for each of the 4 analytes, thus giving 16 possible locations. Each

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will then also have a value in the paired graph for the second set of fluorophores. This gives a cumulative signal measurement non-degenerately indicates the presence or absence of the at least seven polynucleotide analytes (e.g., eight targets), in any combination of presence or absence.

Analyte	FAM	HEX	Cy5	Cy5.5
PLAU	1	0	0	0
CCND1	2	0	0	0
VCL	0	1	0	0
REN	0	2	0	0
BRCA1	0	0	1	0
KLF8	0	0	2	0
SORL1	0	0	0	1
PTEN	0	0	0	2

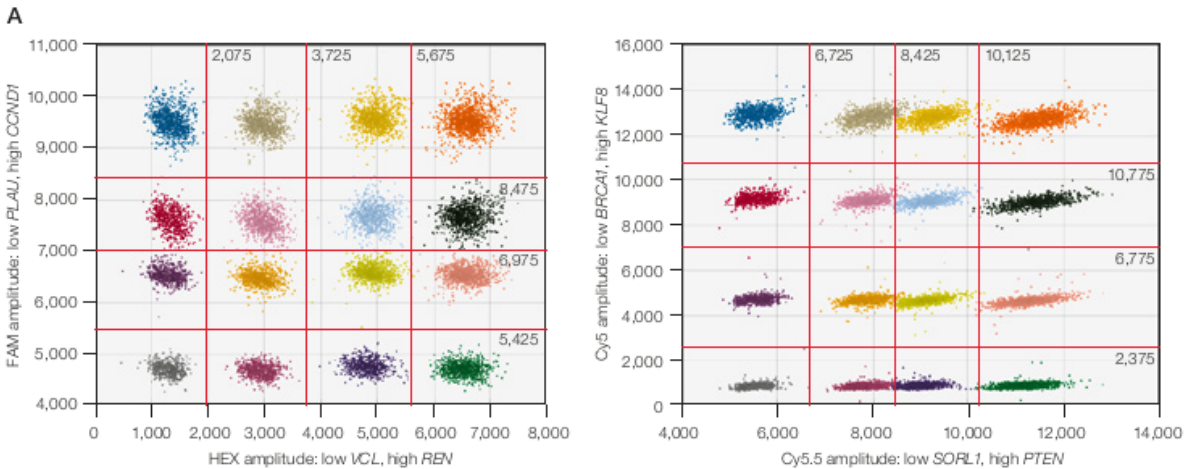
See also:

Table 19. Assay types	
Assay type	Description
Single target per channel	Method assuming up to six probe colors (FAM/EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590) and up to six targets per channel. This is the default assay type, with the maximum six rows displayed. You can delete up to three rows.
Amplitude multiplex	Method to increase multiplexing up to twelve 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 three 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. Group 3 has 1 target in ROX, 1 target in ATTO 590, and one target in both groups. You can delete a group, but not the individual targets within the group. Note: The channels within each 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.

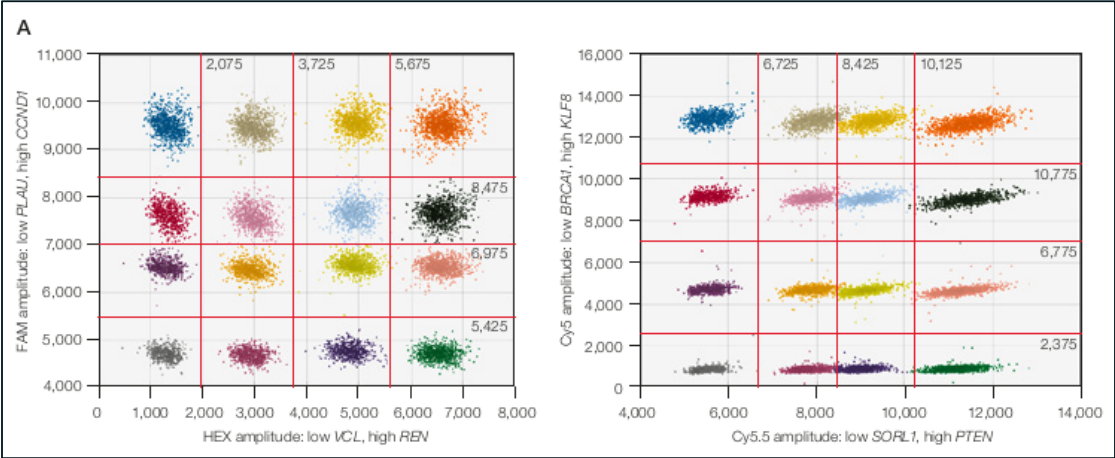
Claims	Exemplary Disclosure of Accused Product
	BIO_CC00007427 at BIO_CC00007505 The QX600 system is used to perform different experiment and assay types to generate a cumulative signal measurement, such as amplitude multiplex and probe mix triplex experiments and assays.
[1d.1] wherein said method non-degenerately detects the presence or absence of the at least seven polynucleotide analytes in said single sample solution volume, in any combination of presence or absence	Plaintiffs incorporate their discussion from steps 1a, 1b, and 1c in their entirety. As discussed in those steps, the presence or absence of the at least seven polynucleotide analytes the sample is detected non-degenerately. Further: The QX600 Droplet Digital PCR System is used to perform the method non-degenerately detects the presence or absence of the at least seven polynucleotide analytes in said single sample solution volume, in any combination of presence or absence, both literally and under the doctrine of equivalents. SENSITIVE MULTIPLEXING ▪ 6-color detection capability (FAM/EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590) ▪ Quantification of 12 targets in a single well ▪ Absolute quantification with 0.1% or better sensitivity BIO_CC00008190 at 8191

Claims	Exemplary Disclosure of Accused Product												
	<table><tr><th colspan="2">Table 19. Assay types</th></tr><tr><th>Assay type</th><th>Description</th></tr><tr><td>Single target per channel</td><td>Method assuming up to six probe colors (FAM/EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590) and up to six targets per channel. This is the default assay type, with the maximum six rows displayed. You can delete up to three rows.</td></tr><tr><td>Amplitude multiplex</td><td>Method to increase multiplexing up to twelve 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.</td></tr><tr><td>Probe mix triplex</td><td>Triplex mode that allows three 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. Group 3 has 1 target in ROX, 1 target in ATTO 590, and one target in both groups. You can delete a group, but not the individual targets within the group. Note: The channels within each group are not interchangeable therefore FAM is always paired with HEX/VIC and so forth.</td></tr><tr><td>Advanced classification</td><td>Method to increase multiplexing rate in any combination of targets detected per channel. This method requires manual classification of droplets.</td></tr></table> BIO_CCODE00007427 at 7505. The QX600 is used to quantify at least seven targets in a single well with absolute quantification with 0.1% or better sensitivity (<i>e.g.</i>, non-degenerately).	Table 19. Assay types		Assay type	Description	Single target per channel	Method assuming up to six probe colors (FAM/EvaGreen®, HEX/VIC, Cy5, Cy5.5, ROX, and ATTO 590) and up to six targets per channel. This is the default assay type, with the maximum six rows displayed. You can delete up to three rows.	Amplitude multiplex	Method to increase multiplexing up to twelve 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 three 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. Group 3 has 1 target in ROX, 1 target in ATTO 590, and one target in both groups. You can delete a group, but not the individual targets within the group. Note: The channels within each 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.
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Advanced classification	Method to increase multiplexing rate in any combination of targets detected per channel. This method requires manual classification of droplets.												

Claims	Exemplary Disclosure of Accused Product
	Setting Up Experiment Parameters To set experiment parameters 1. Open the analysis data file and select the Plate Editor tab. 2. Select one or more wells to be assigned well analysis attributes. 3. Change the experiment type to Direct Quantification (DQ), and then click Apply. 4. (Optional) Change the sample description identifiers. 5. (Optional) Change the sample type. The default type is Unknown. Tip: For automatic thresholding of positive control wells, you must select Pos Ctrl. 6. Under Assay Type, select Amplitude Multiplex and click Apply. Twelve target rows appear. 7. Change the target name and type (unknown or reference) for each target in the well. The software defaults to FAM Lo, FAM Hi, HEX Lo, HEX Hi, Cy5 Lo, Cy5 Hi, Cy5.5 Lo, Cy5.5 Hi, ROX Lo, ROX Hi, ATTO 590 Lo, and ATTO 590 Hi, respectively, for each target. 8. (Optional) Change the high and low target identifiers. Amplitude Multiplex allows for up to two assays in each channel, indicated by Lo and Hi labels. In this multiplex mode, each target may have a signal for only one channel. You can ■ Select a different dye, if the channel supports two dyes (for example change HEX to VIC or VIC to HEX) ■ Select a different channel for the target (for example, change FAM to HEX) ■ Change Hi to Lo or Lo to Hi 9. Click Apply. BIO CCODE00007427 at 7612. The QX600 System defaults to FAM Lo, FAM Hi, HEX Lo, HEX Hi, Cy5 Lo, Cy5 Hi, Cy5.5 Lo, Cy5.5 Hi, ROX Lo, ROX Hi, ATTO 590 Lo, and ATTO 590 Hi, respectively, for each target. Thus, on information and belief, each target is encoded with a specific fluorophore corresponding to a cumulative signal measurement to non-degenerately indicate the presence or absence of each target.

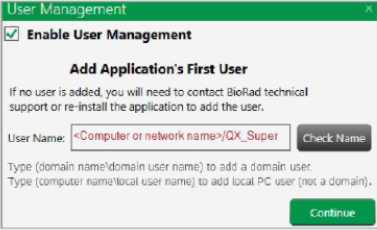
Claims	Exemplary Disclosure of Accused Product
	A  BIO_CC000008190 at 8192. Plaintiffs incorporate their discussion about this exemplary plot from Step 1c.2 here by reference.
[1d.2] without requiring any step of immobilization of said polynucleotide analytes, physical separation of said polynucleotide	Plaintiffs incorporate their discussion from steps 1a, 1b, and 1c in their entirety. As discussed in those steps, the presence or absence of the at least seven polynucleotide analytes the sample is detected non-degenerately. Also, as shown above, no immobilizing or physical separation of any polynucleotide analytes occurs. Likewise, no mass spectrometry is performed. For example:

Claims	Exemplary Disclosure of Accused Product
analytes, or mass spectrometry.	Direct Quantification with the Advanced Classification Method The Advanced Classification Method analysis mode allows the user complete control of droplet identification, and does not force or preassign any clusters. This allows a higher degree of multiplexing through radial dye mixing. Optionally, to expedite plate setup you can copy and paste data from one well to another, and you can click Undo to undo an applied well setting. Important: This mode is intended for experienced users of Droplet Digital PCR. BIO_CCODE00007427 at 7621. The assay does not require these techniques as it does not force or preassign any clusters by immobilization of said polynucleotide analytes.
2. The method of claim 1, wherein said hybridization probes comprise an identical fluorophore, and wherein said plurality of hybridization probes are specific for different polynucleotide analytes.	Plaintiffs incorporate their analysis from Claim 1 here. The QX600 Droplet Digital PCR System is used to perform the step that hybridization probes comprise an identical fluorophore, and wherein said plurality of hybridization probes are specific for different polynucleotide analytes, both literally and under the doctrine of equivalents. The following exemplary plot shows the hybridization probes comprising identical fluorophores but also being specific for different polynucleotide analytes:

Claims	Exemplary Disclosure of Accused Product
	 BIO_CC000008190 at 8192. As seen above, the hybridization probe corresponding to the PLAUI analyte comprises the FAM fluorophore. The hybridization probe corresponding to the CCND1 analyte comprises an identical FAM fluorophore as well. The intensity differences (<i>e.g.</i>, low FAM signal for PLAUI and high FAM signal for CCND1) for an identical fluorophore (<i>e.g.</i>, FAM) allows for said plurality of hybridization probes comprising an identical fluorophore to be contained in a probe that is specific for different polynucleotide analytes.
3. The method of claim 1, wherein said detecting is performed with a plurality of hybridization probes comprising different fluorophores, wherein said plurality of	Plaintiffs incorporate by reference their discussion from Claim 1, including in particular claimed steps 1a. As discussed there, the QX600 is designed to with a plurality of hybridization probes comprising different fluorophores, wherein said plurality of hybridization probes are specific for different analytes, both literally and under the doctrine of equivalents. For example:

Claims	Exemplary Disclosure of Accused Product
hybridization probes are specific for different analytes.	BIO_CC00007427 at 7618 As shown in the example figure above, each probe corresponds to one of the polynucleotide analytes: the NHEJ probe corresponds to NHEJ, and the reference probe corresponds to the reference. Each of the probes also corresponds to a specific fluorophore or a combination of fluorophores (e.g., NHEJ Probe = HEX and Reference Probe = FAM, or NHEJ Probe = FAM and Reference Probe = HEX), with each corresponding to a color indicated in the figure. Thus, the detection is performed with hybridization probes comprising different fluorophores, wherein the hybridization probes are specific for different analytes.
4. The method of claim 1, wherein said cumulative intensity signal(s) are generated during a polymerase chain reaction.	Plaintiffs incorporate their analysis from Claim 1. That analysis describes that the QX600 ddPCR system performs a polymerase chain reaction (PCR). For example: Droplet Digital™ PCR (ddPCR™) is a digital polymerase chain reaction method based on water-oil emulsion droplet technology. ddPCR uses a combination of microfluidics and proprietary surfactant chemistries to divide each sample into water-in-oil droplets. The technology uses reagents and workflows similar to those used for most standard TaqMan probe-based assays, and provides absolute quantification of nucleic acid target sequences by counting nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions.

Claims	Exemplary Disclosure of Accused Product
	BIO_CC00007427 at 7449. Droplet digital PCR is a digital polymerase chain reaction method. The QX600 system uses hydrolysis probes designed to generate signals during a polymerase chain reaction, and cumulative intensity signals generated during a polymerase chain reaction is a standard step in such techniques. On information and belief, the QX600 is used to create such polymerase chain reactions which, in turn, generate the cumulative intensity signals.
5. The method of claim 1, further comprising transmitting information concerning the presence or absence of said polynucleotide analytes through a computer network.	The QX600 Droplet Digital PCR System is used to perform the step of transmitting information concerning the presence or absence of said polynucleotide analytes through a computer network, both literally and under the doctrine of equivalents. The QX600 is designed to be operated over a network:

Claims	Exemplary Disclosure of Accused Product
	Enabling User Management During the installation of QX Manager Software, Standard Edition, you can choose to enable or bypass the User Management module: ■ If the Enable User Management checkbox is selected, all user functions are included in the installation. ■ If the checkbox is not selected, User Management is not enabled and the corresponding tab is not included in the side pane. A single generic user, with a small set of user preferences, is always logged in. For information, see Partial Preferences on page 44. Important: Before you can set up users in the software, all users must be set up on the computer as local Windows users or in Active Directory on your network.  To bypass the User Management module ► Ensure the checkbox is cleared and click Continue in the User Management dialog box to complete the installation. To enable the User Management module 1. Ensure the Enable User Management checkbox is selected. 2. Enter the user name and click Check Name to validate the user. 3. Click Continue to add the user and complete the installation. BIO_CCODE00007427 at 7463.

Claims

Exemplary Disclosure of Accused Product

POWERFUL SOFTWARE

- Results that are easy to analyze
- Premium edition available featuring audit trails and tools for U.S. FDA 21 CFR Part 11 compliance
- Premium edition supports assay protocol files for automated data analysis and reporting

BIO_CCODE00008191.

Table 40. Example User Roles, continued

Privilege	Superuser	Lab Manager	Group Lead	Technician/Student Intern
Archive data files	✓	✓	✓	✓
Access the module recovery tool	✓	✓	✓	✓
Manage Plates	✓	✓	✓	

You can assign the Create new templates privilege to non-administrative roles if anyone can create and save a template. If template formats are restricted, then you should assign this permission to administrative or supervisory roles only.

Note: Bio-Rad recommends defining at least one additional superuser as a backup, as well as other administrative and standard roles based on your needs. Guest users can open shared templates, execute runs, and perform analysis but are not typically assigned other privileges.

To add or modify privileges

1. Click the Add/Manage Users tab to open the User Management window.
2. Select a user from the Current Users list. The name appears in the User Name field. A **network** or computer name and backslash might appear before the user name.
3. Select or clear the checkbox for each privilege in accordance with the user's role in using the software. You can assign user privileges in any combination.
4. When the confirmation message appears, click Yes to save the changes, and then click OK to close the pop-up.

BIO_CCODE00007427 at 7609

Claims	Exemplary Disclosure of Accused Product
	On information and belief, the QX600 system performs the data collection and analysis of the system through a computer network and also uses a computer software. It transmits information concerning the presence or absence of said polynucleotide analytes through a computer network
6. The method of claim 1, further comprising providing information concerning the presence or absence of said polynucleotide analytes to a health care professional, wherein the health care professional makes a clinical decision based on said information.	On information and belief, after using the QX600, Bio-Rad and/or an end-user provides the information concerning the presence or absence of said polynucleotide analytes to a health care professional, wherein the health care professional makes a clinical decision based on said information, both literally and under the doctrine of equivalents. For example:

Claims	Exemplary Disclosure of Accused Product
	ddPCR is highly effective in the following areas: ■ Absolute quantification — ddPCR provides a concentration of target DNA copies per input sample without the need for running standard curves, making this technique ideal for target DNA measurements, viral load analysis, and microbial quantification. ■ Genomic alterations such as gene copy number variation (CNV) — CNVs result in too few or too many dosage-sensitive genes responsible for phenotypic variability, complex behavioral traits, and disease. ddPCR enables measurement of 1.2x differences in gene copy number. ■ Detection of rare sequences — researchers must amplify single genes in a complex sample, such as a few tumor cells in a wild-type background. ddPCR is sensitive enough to detect rare mutations or sequences. ■ Gene expression and microRNA analysis — ddPCR provides stand-alone absolute quantification of expression levels, especially low-abundance microRNAs, with sensitivity and precision. ■ Next-generation sequencing (NGS) — ddPCR quantifies NGS sample library preparations to increase sequencing accuracy and reduce run repeats. Validate sequencing results such as single nucleotide polymorphisms or copy number variations with absolute quantification. ■ Single cell analysis — the high degree (10-fold to 100-fold) of cell-cell variation in gene expression and genomic content among homogeneous post-mitotic, progenitor, and stem cell populations drives a need for analysis from single cells. ddPCR enables low copy number quantification and gene expression of individual cells. ■ Genome edit detection — ddPCR enables fast, precise, and cost-effective assessment of HDR (Homology directed repair) and NHEJ (non-homologous end joining) generated by CRISPR-Cas9 or other genome editing tools. BIO_CC00007427 at BIO_CC00007449

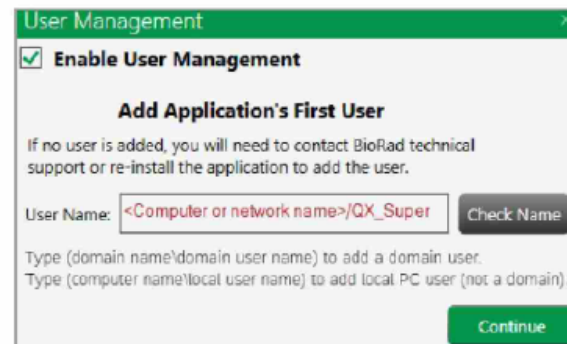
Claims	Exemplary Disclosure of Accused Product
	The QX600 system can be used for detection of rare sequences, analyzing genomic alterations, quantification of gene expression and microRNA analysis, etc., which can provide information concerning the presence or absence of said analytes to a health care professional, wherein the health care professional makes a clinical decision based on said information.
7. The method of claim 1, wherein at least one step of said method is performed using a computer.	Plaintiffs incorporates by reference Claims 1 and 5. <i>See also</i> at least Chapters 6-9 of the QX600 user guide describing operations used with a computer. The QX600 Droplet Digital PCR System is used to perform the step of wherein at least one step of said method is performed using a computer, both literally and under the doctrine of equivalents. For example:

Enabling User Management

During the installation of QX Manager Software, Standard Edition, you can choose to enable or bypass the User Management module:

- If the Enable User Management checkbox is selected, all user functions are included in the installation.
- If the checkbox is not selected, User Management is not enabled and the corresponding tab is not included in the side pane. A single generic user, with a small set of user preferences, is always logged in. For information, see [Partial Preferences on page 44](#).

Important: Before you can set up users in the software, all users must be set up on the computer as local Windows users or in Active Directory on your network.



To bypass the User Management module

- Ensure the checkbox is cleared and click Continue in the User Management dialog box to complete the installation.

To enable the User Management module

1. Ensure the Enable User Management checkbox is selected.
2. Enter the user name and click Check Name to validate the user.
3. Click Continue to add the user and complete the installation.

Claims	Exemplary Disclosure of Accused Product
	BIO_CCODE00007427 at BIO_CCODE00007463 POWERFUL SOFTWARE ▪ Results that are easy to analyze ▪ Premium edition available featuring audit trails and tools for U.S. FDA 21 CFR Part 11 compliance ▪ Premium edition supports assay protocol files for automated data analysis and reporting BIO_CCODE00008190 at BIO_CCODE00008191

Claims	Exemplary Disclosure of Accused Product
	Chapter 3 About QX Manager Software QX Manager Software, when connected to your QX600 Droplet Reader, provides the necessary functionality to create, run, and analyze droplet reading experiments on your samples. During the installation of Standard Edition, you can enable the User Management module for all user management functions and preferences. If you do not enable User Management, you can use the software with a single generic user. Using the instrument and software, you can ■ Set up customized, real-time ddPCR experiments for plate runs ■ Read standard droplets ■ Choose between two droplet volumes ■ See the available disk space and number of plates you can process ■ Use the live analysis function during the droplet reading process ■ Analyze your data files in a variety of charts and tables in the Analysis module ■ Reprocess data with updated color calibration values ■ Create and store plate and report templates, and produce reports on your data BIO_CCODE00007427 at BIO_CCODE00007461 The QX600 system uses a separate analytical software for the analysis of the data collected during the performance of the method. This software, QX Manager, specifically is used to define the well, targets, fluorophores and intensities of the method and provides the final calculations of concentration, ratio, and fractional abundance as well as data visualization options.

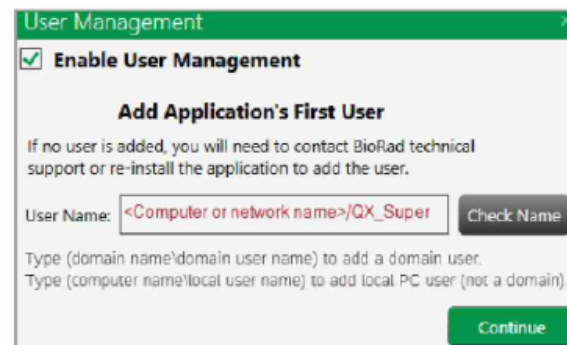
Claims	Exemplary Disclosure of Accused Product
	Also, as discussed in Claim 1, a thermal cycler is used in multiple steps. A thermal cycler may have a computer. Also, as discussed in Claim 1, the QX600 Droplet Reader may be the computer.
8. The method of claim 7, wherein said computer is located on a remote server.	Plaintiffs incorporates by reference their analysis in Claims 5 and 7. The QX600 Droplet Digital PCR System is used to perform the step of said computer is located on a remote server, both literally and under the doctrine of equivalents. For example:

Enabling User Management

During the installation of QX Manager Software, Standard Edition, you can choose to enable or bypass the User Management module:

- If the Enable User Management checkbox is selected, all user functions are included in the installation.
- If the checkbox is not selected, User Management is not enabled and the corresponding tab is not included in the side pane. A single generic user, with a small set of user preferences, is always logged in. For information, see [Partial Preferences on page 44](#).

Important: Before you can set up users in the software, all users must be set up on the computer as local Windows users or in Active Directory on your network.



To bypass the User Management module

- Ensure the checkbox is cleared and click Continue in the User Management dialog box to complete the installation.

To enable the User Management module

1. Ensure the Enable User Management checkbox is selected.
2. Enter the user name and click Check Name to validate the user.
3. Click Continue to add the user and complete the installation.

Claims	Exemplary Disclosure of Accused Product
	BIO_CCODE00007427 at BIO_CCODE00007463 Typically, a system or software administrator sets up users and privileges for QX Manager Software, and communicates the information to you. Note the following: ■ When you sign in the first time after the software is installed, you must select the instrument to which the computer is connected. See Selecting the Instrument on page 40 for information. BIO_CCODE00007427 at BIO_CCODE00007465 The QX600 system instrument can be used on a network and can be selected from a connected computer which can be located on a remote server.
9. The method of claim 7, wherein said computer is located on a thermal cyclor.	Plaintiffs incorporate their analysis from Claims 1 and 7 here. As discussed in Claim 1, multiple steps of the claim can be performed on a thermal cyclor which can have a computer. The QX600 Droplet Digital PCR System is used to perform the step of said computer is located on a thermal cyclor, both literally and under the doctrine of equivalents. For example:

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. BIO_CCODE00007427 at BIO_CCODE00007450 Thermal Cycling Conditions Follow instructions based on the system in use: ■ For the QX600 or QX200 Droplet Digital PCR System, after droplet generation with the QX200 Droplet Generator, carefully transfer droplets into a clean 96-well plate. Seal the plate using the PX1 PCR Plate Sealer (#1814000) at 180°C for 5 sec. Proceed to thermal cycling (see Table 2) ■ For the QX600 or QX200 AutoDG Droplet Digital PCR System, remove the droplet plate containing ddPCR droplets from the Automated Droplet Generator. Seal the plate using the PX1 PCR Plate Sealer at 180°C for 5 sec. Proceed to thermal cycling (see Table 2)

Claims	Exemplary Disclosure of Accused Product															
	BIO_CC00007878 Important: Your QX600 Droplet Reader performs the post-PCR droplet reading function only. A droplet generator and a thermal cycler are required to perform a full ddPCR cycle. You can purchase a droplet generator and thermal cycler, as well as associated accessories and consumables, from Bio-Rad. For information, see Appendix D, Ordering Information. BIO_CC00007427 at BIO_CC00007451 Table 7. Thermal cycler and plate sealer <table><tr><th>Product</th><th>Description</th><th>Catalog Number</th></tr><tr><td>C1000™ Touch Thermal Cycler with 96–Deepwell Reaction Module</td><td>Includes the C1000 Touch Thermal Cycler chassis, 96–deepwell reaction module, USB flash drive</td><td>1851197</td></tr><tr><td>PTC Tempo Deepwell Thermal Cycler</td><td>Includes the thermal cycler, power cord, USB cable, and Ethernet cable</td><td>12015392</td></tr><tr><td>PX1 PCR Plate Sealer</td><td>PCR plate sealer, includes heat sealing instrument, plate support block that holds 96-well and 384-well plates, sealing frame, power cord</td><td>1814000</td></tr><tr><td>Piercable Foil Heat Seal</td><td>Heat seal for the PX1 PCR Plate Sealer</td><td>1814040</td></tr></table> BIO_CC00007427 at 7456. The OX600 system uses a thermal cycler to perform ddPCR workflow.	Product	Description	Catalog Number	C1000™ Touch Thermal Cycler with 96–Deepwell Reaction Module	Includes the C1000 Touch Thermal Cycler chassis, 96–deepwell reaction module, USB flash drive	1851197	PTC Tempo Deepwell Thermal Cycler	Includes the thermal cycler, power cord, USB cable, and Ethernet cable	12015392	PX1 PCR Plate Sealer	PCR plate sealer, includes heat sealing instrument, plate support block that holds 96-well and 384-well plates, sealing frame, power cord	1814000	Piercable Foil Heat Seal	Heat seal for the PX1 PCR Plate Sealer	1814040
Product	Description	Catalog Number														
C1000™ Touch Thermal Cycler with 96–Deepwell Reaction Module	Includes the C1000 Touch Thermal Cycler chassis, 96–deepwell reaction module, USB flash drive	1851197														
PTC Tempo Deepwell Thermal Cycler	Includes the thermal cycler, power cord, USB cable, and Ethernet cable	12015392														
PX1 PCR Plate Sealer	PCR plate sealer, includes heat sealing instrument, plate support block that holds 96-well and 384-well plates, sealing frame, power cord	1814000														
Piercable Foil Heat Seal	Heat seal for the PX1 PCR Plate Sealer	1814040														

Appendix A-2: Infringement Claim Chart for U.S. Patent No. 10,068,051 and QX ONE

Use of Bio-Rad's QX ONE Droplet Digital PCR (ddPCR) System ("QX ONE") (includes both standard and premium editions) infringes Claims 1-9 of U.S. Patent No. 10,068,051 ("the '051 Patent"). As non-limiting examples, Plaintiffs identify where and how use of the Accused Product performs each and every step of the below claimed method. Elements from other charts may be used in combination with the below to perform the steps of the claimed method, and Plaintiffs incorporate all other '051 Charts here by reference.

As non-limiting examples, Plaintiffs identify where and how use of the Accused Product performs each and every step of the below claimed method. Elements from other charts may be used in combination with the below to perform the steps of the claimed method, and Plaintiffs incorporate all other '051 Charts here by reference.

On information and belief, the QX600 is used with devices, accessories, and components to practice the methods of the '051 Patent. See <https://www.bio-rad.com/en-us/life-science/digital-pcr/qx-one-droplet-digital-pcr-ddpcr-system>. 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 Standard editions);
- Consumables such as
 - o GCR96 Cartridges,
 - o QX ONE Droplet Generation Oil for Probes,
 - o QX ONE Droplet Reader Oil,
 - o GCR96 Foil Heat Seal, and/or
 - o QX ONE ddPCR System Waste Bottle;
- A plate sealer, such as the PX1 PCR Plate Sealer;
- ddPCR kits and reagents, such as
 - o PrimePCR ddPCR Assays,
 - o ddPCR Multiplex Supermix,
 - o ddPCR Supermixes for Probes (No dUTP),
 - o ddPCR Supermix for Probes,
 - o One-Step RT-ddPCR Advanced Kit for Probes,
 - o ddPCR Buffer Control for Probes,
 - o Residual DNA Quantification Supermixes,
 - o QX200 ddPCR EvaGreen Supermix,

- o QX200 Buffer Control for EvaGreen, and
 - o ddPCR Library Quantification Kit for Illumina TruSeq;
- Consumables, such as
 - o the ddPCR 96-Well Plates,
 - o Droplet Generation Oil for Probes,
 - o QX200 Droplet Generation Oil for EvaGreen,
 - o Droplet Reader Oil,
 - o DG8 Cartridges and DG8 Gaskets,
 - o DG8 Cartridge Holders,
 - o pierceable foil such as the PCR Plate Heat Seal,
 - o DG32 Cartridges for AutoDG,
 - o Pipet Tips for AutoDG,
 - o Pipet Tip Waste Bin for AutoDG;
- PrimePCR Primers, Assays and Arrays;
- Each of the assays identified in these Infringement Contentions; and/or
- Any other processes, procedures, devices, and/or system components used to facilitate the performance of the steps of the Asserted Claims.

Bio-Rad directly infringes the Asserted Claims by using the QX One alone and/or with the above-listed devices, software, accessories, and/or components to perform each and every step of the claim methods below. By way of non-limiting example, Bio-Rad uses the QX One in connection with (but not exclusively with) the various assays identified in these Infringement Contentions to practice each and every step of the Asserted Claims.

Bio-Rad also indirectly infringes the Asserted Claims by providing the QX One alone and/or with the accompanying QX One Software. Bio-Rad also induces customers and/or end users to use the QX One and/or QX One Software to infringe by inducing them to use these products in a way that performs each step 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, such customers and/or end users use the QX One in connection with (but not exclusively with) the various assays identified in these Infringement Contentions to practice each and every step of the Asserted Claims.

Bio-Rad also contributes to the infringement of customers and/or end-users by providing the QX One and/or the QX One Software to the same who then perform each step 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 in connection with (but not

exclusively with) the various assays identified in these Infringement Contentions to practice each and every step 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 QX One Software such that Bio-Rad induces infringe 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).

On information and belief, to the extent Bio-Rad performs the steps of the Asserted Claims outside the United States and subsequently imports, offers to sell, sells, and/or uses the results of the claimed methods within the United States, Bio-Rad infringes in violation of 35 U.S.C. § 271(g).

Plaintiffs allege infringement both literally and under the doctrine of equivalents.

Claims	Exemplary Disclosure of Accused Product
[1-PRE] 1. A method of detecting the presence or absence of at least seven polynucleotide analytes, in any combination of presence or absence, in a single sample solution volume, the method comprising:	To the extent the preamble is found to be limiting, the QX One System is used to perform a method of detecting the presence or absence of at least seven polynucleotide analytes, in any combination of presence or absence, in a single sample solution volume both literally and under the doctrine of equivalents. The QX One System can perform different types of experiments which includes multiplex assays to quantify up to 8 targets (analytes) with 4 colors must include the steps of identifying the presence or absence of at least 7 of those targets. The system allows for clear discrimination of multiple targets and more accurate and reproducible quantification, which allows it to detect the presence or absence of polynucleotide analytes with high accuracy, precision, and reproducibility. The QX ONE System comprises multiple components and kits.

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. QX ONE Droplet Digital PCR System and QX ONE Software Instrument Guide Version 1.4, BIO_CC00006554 at 6577.

Claims	Exemplary Disclosure of Accused Product																
	Experiment Types QX ONE Software offers the experiment types described in Table 9, each enabling different assay options and analysis tools. You can assign multiple experiment types across the plate, but you can assign only one experiment type to each well. Table 9. Experiment types <table><tr><th>Type</th><th>Description</th></tr><tr><td>Direct quantification (DQ)</td><td>DQ uses absolute quantification to determine the concentration (copies/μl) of target DNA copies in a sample. DQ measures the number of positive and negative droplets for each target in a well based on their fluorescence amplitude, and uses a Poisson algorithm to calculate the starting concentration of each target DNA molecule. Note: The ABS experiment type created in QuantaSoft Software versions 1.4 through 1.7 is automatically mapped into the software as a DQ experiment type.</td></tr><tr><td>Copy number variation (CNV)</td><td>CNV determines concentration (as with DQ) and calculates the copy number of an unknown (CNV) target relative to a known reference or references within the same well.</td></tr><tr><td>Mutation detection (MUT)</td><td>MUT determines concentration (as with DQ) and calculates the fractional abundance of an unknown mutant present at a low frequency in a wild-type background.</td></tr><tr><td>Rare event detection (RED)</td><td>RED determines the concentration of a known mutant or a rare target species relative to a given reference species within a large pool of background DNA.</td></tr><tr><td>Drop-off (DOF)</td><td>DOF determines absolute quantification of targets for assays designed to detect non-wild type sequences, such as indels and genome edits; DOF is designed to support an assay strategy where one probe counts all alleles and one "drop-off" probe sits on top of a predicted cut site or mutation site.</td></tr><tr><td>Gene expression (GEX)</td><td>GEX determines concentration (as with DQ) and calculates relative expression levels of an unknown target relative to a known reference or references within the same well.</td></tr><tr><td>Residual DNA quantification (RDQ)</td><td>RDQ provides a precise method for residual host cell DNA quantification. Note: Users can enter a conversion factor to calculate the mass for a well, up to three decimals. The software displays the result in the Molecular Weight column of the Data Table.</td></tr></table> BIO_CC00006618. The QX One System can detect up to eight targets using four fluorophores.	Type	Description	Direct quantification (DQ)	DQ uses absolute quantification to determine the concentration (copies/ μ l) of target DNA copies in a sample. DQ measures the number of positive and negative droplets for each target in a well based on their fluorescence amplitude, and uses a Poisson algorithm to calculate the starting concentration of each target DNA molecule. Note: The ABS experiment type created in QuantaSoft Software versions 1.4 through 1.7 is automatically mapped into the software as a DQ experiment type.	Copy number variation (CNV)	CNV determines concentration (as with DQ) and calculates the copy number of an unknown (CNV) target relative to a known reference or references within the same well.	Mutation detection (MUT)	MUT determines concentration (as with DQ) and calculates the fractional abundance of an unknown mutant present at a low frequency in a wild-type background.	Rare event detection (RED)	RED determines the concentration of a known mutant or a rare target species relative to a given reference species within a large pool of background DNA.	Drop-off (DOF)	DOF determines absolute quantification of targets for assays designed to detect non-wild type sequences, such as indels and genome edits; DOF is designed to support an assay strategy where one probe counts all alleles and one "drop-off" probe sits on top of a predicted cut site or mutation site.	Gene expression (GEX)	GEX determines concentration (as with DQ) and calculates relative expression levels of an unknown target relative to a known reference or references within the same well.	Residual DNA quantification (RDQ)	RDQ provides a precise method for residual host cell DNA quantification. Note: Users can enter a conversion factor to calculate the mass for a well, up to three decimals. The software displays the result in the Molecular Weight column of the Data Table.
Type	Description																
Direct quantification (DQ)	DQ uses absolute quantification to determine the concentration (copies/ μ l) of target DNA copies in a sample. DQ measures the number of positive and negative droplets for each target in a well based on their fluorescence amplitude, and uses a Poisson algorithm to calculate the starting concentration of each target DNA molecule. Note: The ABS experiment type created in QuantaSoft Software versions 1.4 through 1.7 is automatically mapped into the software as a DQ experiment type.																
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Rare event detection (RED)	RED determines the concentration of a known mutant or a rare target species relative to a given reference species within a large pool of background DNA.																
Drop-off (DOF)	DOF determines absolute quantification of targets for assays designed to detect non-wild type sequences, such as indels and genome edits; DOF is designed to support an assay strategy where one probe counts all alleles and one "drop-off" probe sits on top of a predicted cut site or mutation site.																
Gene expression (GEX)	GEX determines concentration (as with DQ) and calculates relative expression levels of an unknown target relative to a known reference or references within the same well.																
Residual DNA quantification (RDQ)	RDQ provides a precise method for residual host cell DNA quantification. Note: Users can enter a conversion factor to calculate the mass for a well, up to three decimals. The software displays the result in the Molecular Weight column of the Data Table.																

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 <table> <tr> <th>Assay type</th><th>Description</th></tr> <tr> <td>Single target per channel</td><td>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.</td></tr> <tr> <td>Amplitude multiplex</td><td>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.</td></tr> <tr> <td>Probe mix triplex</td><td>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.</td></tr> <tr> <td>Advanced classification</td><td>Method to increase multiplexing rate in any combination of targets detected per channel. This method requires manual classification of droplets.</td></tr> <tr> <td>Basic drop-off</td><td>This mode allows two groups of two targets each. The first group appears by default, with one target in FAM <u>and</u> HEX/VIC and one target in FAM <u>or</u> 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 <u>and</u> Cy5.5, one target in Cy5 <u>or</u> 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.</td></tr> </table>	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 <u>and</u> HEX/VIC and one target in FAM <u>or</u> 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 <u>and</u> Cy5.5, one target in Cy5 <u>or</u> 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.
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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 <u>and</u> HEX/VIC and one target in FAM <u>or</u> 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 <u>and</u> Cy5.5, one target in Cy5 <u>or</u> 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.												

Claims

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Table 10. Assay types, continued

Assay type	Description
Double drop-off	This assay type is available only for the DOF experiment type, and allows two groups of three targets each. The first group appears by default, and looks for one target in FAM and in HEX/VIC (target type reference) one target in FAM (target type unknown), and one target in HEX/VIC (target type unknown). You can add the second group to look for one target in Cy5 and Cy5.5 (target type reference, with two reference copies), one target in Cy5 (target type unknown), and one target in Cy5.5 (target type unknown). By default, all reference copies are set to 2. The channels are static for each group. (Channel 1 FAM is always paired with Channel 2 HEX/VIC, Channel 3 Cy5 is always paired with Channel 4 Cy5.5.)

The experiment type you select determines the available assay methods. [Table 11](#) displays the sample type associated with each assay method.

Table 11. Assay types by experiment type

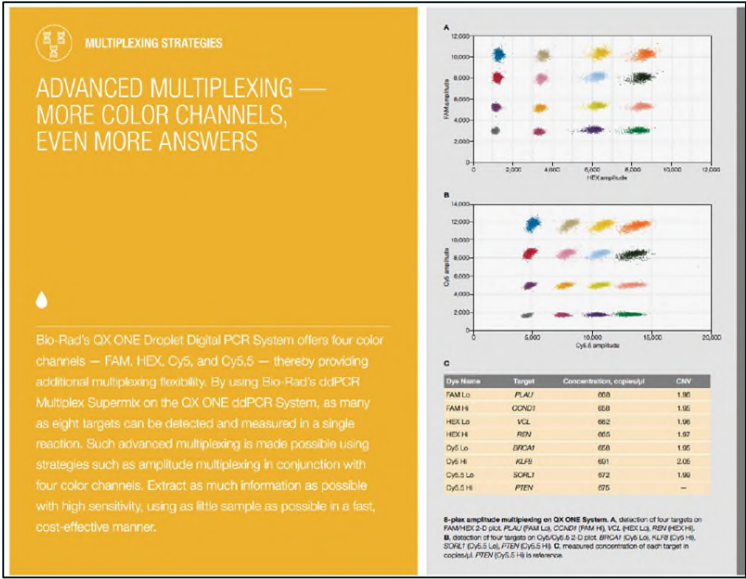
Assay type	Experiment type						
	DQ	CNV	MUT	DOF	GEX	RDQ	RED
Single target per channel	✓	✓	✓		✓	✓	✓
Amplitude multiplex	✓	✓	✓		✓	✓	✓
Probe mix triplex	✓	✓	✓		✓	✓	✓
Advanced classification	✓						✓
Basic drop-off				✓			
Double drop-off				✓			

QX ONE Software automatically populates the fluorophore in each channel for each target but you can change the default selections.

Claims	Exemplary Disclosure of Accused Product																																																																						
	Table 12. Fluorophore options <table><tr><th>Assay method</th><th>Signal Ch1</th><th>Signal Ch2</th><th>Signal Ch3</th><th>Signal Ch4</th></tr><tr><td rowspan="3">Single target per channel, for 1 to 4 targets</td><td>FAM</td><td>HEX</td><td>Cy5</td><td>Cy5.5</td></tr><tr><td>None</td><td>VIC</td><td>None</td><td>None</td></tr><tr><td></td><td>None</td><td></td><td></td></tr><tr><td rowspan="4">Amplitude multiplex, for 1 to 8 targets</td><td>FAM Hi</td><td>HEX Hi</td><td>Cy5 Hi</td><td>Cy5.5 Hi</td></tr><tr><td>FAM Lo</td><td>HEX Lo</td><td>Cy 5 Lo</td><td>Cy 5.5 Lo</td></tr><tr><td>None</td><td>VIC Hi</td><td>None</td><td>None</td></tr><tr><td></td><td>VIC Lo</td><td></td><td></td></tr><tr><td rowspan="3">Probe mix triplex, for 6 targets</td><td>FAM</td><td>HEX</td><td>Cy5</td><td>Cy5.5</td></tr><tr><td>None</td><td>VIC</td><td>None</td><td>None</td></tr><tr><td></td><td>None</td><td></td><td></td></tr><tr><td rowspan="4">Advanced classification</td><td>FAM</td><td>HEX</td><td>Cy5</td><td>Cy5.5</td></tr><tr><td>+1-10</td><td>1 to 10</td><td>1 to 10</td><td>1 to 10</td></tr><tr><td>None</td><td>VIC</td><td>None</td><td>None</td></tr><tr><td></td><td>1 to 10</td><td></td><td></td></tr><tr><td></td><td></td><td>None</td><td></td><td></td></tr></table>	Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4	Single target per channel, for 1 to 4 targets	FAM	HEX	Cy5	Cy5.5	None	VIC	None	None		None			Amplitude multiplex, for 1 to 8 targets	FAM Hi	HEX Hi	Cy5 Hi	Cy5.5 Hi	FAM Lo	HEX Lo	Cy 5 Lo	Cy 5.5 Lo	None	VIC Hi	None	None		VIC Lo			Probe mix triplex, for 6 targets	FAM	HEX	Cy5	Cy5.5	None	VIC	None	None		None			Advanced classification	FAM	HEX	Cy5	Cy5.5	+1-10	1 to 10	1 to 10	1 to 10	None	VIC	None	None		1 to 10					None		
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Claims

Exemplary Disclosure of Accused Product



BIO_CC00008407.

The QX One System analyzes multiple targets (up to 8 targets) using amplitude multiplexing and probe mixing strategies.

Claims	Exemplary Disclosure of Accused Product
	Data Analysis Module The Data Analysis module functionality allows you to ■ View your droplet data in 1D, 2D, and 3D amplitude □ View thresholds automatically assigned by the software based on the distance of the threshold from the means and standard deviations of the positive and negative clusters □ Manually adjust thresholds or classify droplets using cluster modes □ View data in amplitude plots, histograms, and heat maps □ Apply tilt correction to automatic analysis ■ View charts reflecting calculated data visualizations for concentration, copy number, ratio, and fractional abundance ■ Analyze multiple targets within a well using amplitude multiplexing and probe mixing strategies ■ Detect genomic editing events and non-wild type events with a "drop-off assay" analysis option ■ Visualize and export data with improved flexibility ■ Generate reports for different analysis types BIO_CC00006651
[1a.1] a) providing a sample solution volume comprising or potentially comprising, at least one of at least seven polynucleotide analytes,	The QX One System is used to perform the step of providing a sample solution volume comprising or potentially comprising, at least one of at least seven polynucleotide analytes either literally or under the doctrine of equivalents. For example, An exemplary description of the ddPCR Workflow is described in the QX ONE Instrument Guide, which also describes a process for providing a sample solution:

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. 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. PCR Amplification After droplet generation, the plates are moved through the internal thermal cycler, which performs repeated heating and cooling processes. This amplifies one or more copies of a particular DNA segment, thereby multiplying strands of DNA sequencing into the thousands or millions. Droplet Reader Following PCR amplification of the nucleic acid target in the droplets, the plate moves to the Droplet Reader, which analyzes each droplet individually using a four-color detection system, depending on the number of channels specified in the experiment. The autosampler in the Droplet Reader picks up the droplets from each well in the plate. The droplets are spaced out individually for fluorescence reading and fluorescence is then measured in each droplet. Positive droplets, which contain at least one copy of the target DNA molecule, exhibit increased fluorescence compared to negative droplets and are presented in color in the analysis displays. Negative droplets are presented in grayscale.

Claims	Exemplary Disclosure of Accused Product
	BIOCCODE00006554 at 6573-6574. In the QX ONE, several of these steps for sample provision, including droplet generation and thermal cycling, are performed by the same system: 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. BIOCCODE00006554 at 6576; <i>see also id.</i> at 6600-6625. Samples may be provided by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermixes. <i>See id.</i>

Claims	Exemplary Disclosure of Accused Product
	Samples may also be provided using the QX ONE's droplet generator and thermal cycler. During the thermal cycling, a plate sealer such as the PX1 PCR Plate Sealer is used: Description Use the PX1 PCR Plate Sealer for consistent, uniform sealing across the entire plate, minimizing evaporation during cycling. The small-footprint heat sealer is compatible with a wide range of PCR plates and different types of films and foils. Features and Benefits • Ready to seal in less than 3 min • Support block holds both 96- and 384-well plates • Motorized drawer for insertion of plate and seal • Touch-screen interface allowing single button modification of temperature and time • Easy access to previously stored sealing protocols The intuitive touch-screen interface of the PX1 PCR Plate Sealer shows instrument status, platen temperature, protocol name, set temperature, and time. Sealing protocols can be stored and easily edited. The sealer can be programmed to automatically shut down after a user-defined period of idle time. Touching the display after shutdown will reactivate the display and the sealer will begin heating to the last set temperature. The semi-automated PX1 PCR Plate Sealer provides fast, consistent sealing, removing human variability and increasing reproducibility. Uniform sealing over the whole plate is particularly important for low-volume samples where small amounts of evaporation can have significant effect. In qPCR experiments, differential evaporation from wells can lead to large standard deviations in the number of quantification cycles (Cq). Product Components • Heat sealing instrument • Plate support block for both 96- and 384-well plates • Sealing frame Related Products • Optically Clear Heat Seals, for PCR and real-time PCR applications (1814030) • Permanent Clear Heat Seals, nonpeelable, nonpierceable clear seals for PCR, water bath cycling, and sample disposal (1814035) • Pierceable Foil Heat Seals for PCR and QX200™ Droplet Digital™ PCR System applications (1814040) • Peelable Foil Heat Seals laminate/foil peelable seals for PCR and sample storage (1814045) https://www.bio-rad.com/en-us/sku/1814000-px1-pcr-plate-sealer?ID=1814000. The QX One Instruction Manual describes the use of a plate sealer as “required”. BIOCCODE00006554 at 6600. The QX One system requires dividing each sample into a sample solution volume (e.g., a water-in-oil droplet) with target and background DNA randomly distributed into the droplets and allowing for quantification of up to 8 targets in a single well. Thus, each sample volume can provide at least one of at least seven polynucleotide analytes (e.g., quantification of up to 8 targets). The samples are prepared for

Claims	Exemplary Disclosure of Accused Product												
	ddPCR by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermix. See BIOCCODE00006554 at 6573. The QX One System can detect up to eight targets using four fluorophores. Assay Types and Fluorophores QX ONE Software offers the assay methods explained in Table 10. Table 10. Assay types <table><tr><th>Assay type</th><th>Description</th></tr><tr><td>Single target per channel</td><td>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.</td></tr><tr><td>Amplitude multiplex</td><td>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.</td></tr><tr><td>Probe mix triplex</td><td>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.</td></tr><tr><td>Advanced classification</td><td>Method to increase multiplexing rate in any combination of targets detected per channel. This method requires manual classification of droplets.</td></tr><tr><td>Basic drop-off</td><td>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.</td></tr></table>	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.
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Claims

Exemplary Disclosure of Accused Product

Table 10. Assay types, continued

Assay type	Description
Double drop-off	This assay type is available only for the DOF experiment type, and allows two groups of three targets each. The first group appears by default, and looks for one target in FAM and in HEX/VIC (target type reference) one target in FAM (target type unknown), and one target in HEX/VIC (target type unknown). You can add the second group to look for one target in Cy5 and Cy5.5 (target type reference, with two reference copies), one target in Cy5 (target type unknown), and one target in Cy5.5 (target type unknown). By default, all reference copies are set to 2. The channels are static for each group. (Channel 1 FAM is always paired with Channel 2 HEX/VIC, Channel 3 Cy5 is always paired with Channel 4 Cy5.5..

The experiment type you select determines the available assay methods. [Table 11](#) displays the sample type associated with each assay method.

Table 11. Assay types by experiment type

Assay type	Experiment type						
	DQ	CNV	MUT	DOF	GEX	RDQ	RED
Single target per channel	✓	✓	✓		✓	✓	✓
Amplitude multiplex	✓	✓	✓		✓	✓	✓
Probe mix triplex	✓	✓	✓		✓	✓	✓
Advanced classification	✓						✓
Basic drop-off				✓			
Double drop-off				✓			

QX ONE Software automatically populates the fluorophore in each channel for each target but you can change the default selections.

Claims	Exemplary Disclosure of Accused Product																																																																										
	Table 12. Fluorophore options <table><tr><th>Assay method</th><th>Signal Ch1</th><th>Signal Ch2</th><th>Signal Ch3</th><th>Signal Ch4</th></tr><tr><td rowspan="3">Single target per channel, for 1 to 4 targets</td><td>FAM</td><td>HEX</td><td>Cy5</td><td>Cy5.5</td></tr><tr><td>None</td><td>VIC</td><td>None</td><td>None</td></tr><tr><td></td><td>None</td><td></td><td></td></tr><tr><td rowspan="4">Amplitude multiplex, for 1 to 6 targets</td><td>FAM Hi</td><td>HEX Hi</td><td>Cy5 Hi</td><td>Cy5.5 Hi</td></tr><tr><td>FAM Lo</td><td>HEX Lo</td><td>Cy 5 Lo</td><td>Cy 5.5 Lo</td></tr><tr><td>None</td><td>VIC Hi</td><td>None</td><td>None</td></tr><tr><td></td><td>VIC Lo</td><td></td><td></td></tr><tr><td rowspan="3">Probe mix triplex, for 6 targets</td><td>FAM</td><td>HEX</td><td>Cy5</td><td>Cy5.5</td></tr><tr><td>None</td><td>VIC</td><td>None</td><td>None</td></tr><tr><td></td><td>None</td><td></td><td></td></tr><tr><td rowspan="4">Advanced classification</td><td>FAM</td><td>HEX</td><td>Cy5</td><td>Cy5.5</td></tr><tr><td>+1-10</td><td>1 to 10</td><td>1 to 10</td><td>1 to 10</td></tr><tr><td>None</td><td>VIC</td><td>None</td><td>None</td></tr><tr><td></td><td>1 to 10</td><td></td><td></td></tr><tr><td></td><td></td><td>None</td><td></td><td></td></tr></table>					Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4	Single target per channel, for 1 to 4 targets	FAM	HEX	Cy5	Cy5.5	None	VIC	None	None		None			Amplitude multiplex, for 1 to 6 targets	FAM Hi	HEX Hi	Cy5 Hi	Cy5.5 Hi	FAM Lo	HEX Lo	Cy 5 Lo	Cy 5.5 Lo	None	VIC Hi	None	None		VIC Lo			Probe mix triplex, for 6 targets	FAM	HEX	Cy5	Cy5.5	None	VIC	None	None		None			Advanced classification	FAM	HEX	Cy5	Cy5.5	+1-10	1 to 10	1 to 10	1 to 10	None	VIC	None	None		1 to 10					None		
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Amplitude multiplex, for 1 to 6 targets	FAM Hi	HEX Hi	Cy5 Hi	Cy5.5 Hi																																																																							
	FAM Lo	HEX Lo	Cy 5 Lo	Cy 5.5 Lo																																																																							
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Probe mix triplex, for 6 targets	FAM	HEX	Cy5	Cy5.5																																																																							
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[1a.2] and comprising at least seven hybridization probes, each hybridization probe corresponding to one of the at least seven polynucleotide analytes,	The QX One System is used to perform a method using samples with at least seven hybridization probes, each hybridization probe corresponding to one of the at least seven polynucleotide analytes, either literally or under the doctrine of equivalents. When the QX600 is used, the samples provided comprise at least seven hybridization probes with each hybridization probe corresponding to one of at least seven polynucleotide analytes.																																																																										

Claims	Exemplary Disclosure of Accused Product
	Chapter 1 Introduction to Droplet Digital PCR Droplet Digital™ PCR is a digital polymerase chain reaction method based on water-oil emulsion droplet technology. Droplet Digital PCR™ (ddPCR™) uses a combination of microfluidics and proprietary surfactant chemistries to divide each sample into water-in-oil droplets. The technology uses reagents and workflows similar to those used for most standard TaqMan probe-based assays, and provides absolute quantification of nucleic acid target sequences by counting nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions. ddPCR is highly effective in the following areas: ■ Absolute quantification — ddPCR provides a concentration of target DNA copies per input sample without the need for running standard curves, making this technique ideal for target DNA measurements, viral load analysis, and microbial quantification. ■ Genomic alterations such as gene copy number variation (CNV) — CNVs result in too few or too many dosage-sensitive genes responsible for phenotypic variability, complex behavioral traits, and disease. ddPCR enables measurement of 1.2x differences in gene copy number. ■ Detection of rare sequences — researchers must amplify single genes in a complex sample, such as a few tumor cells in a wild-type background. ddPCR is sensitive enough to detect rare mutations or sequences. ■ Gene expression and microRNA analysis — ddPCR provides stand-alone absolute quantification of expression levels, especially low-abundance microRNAs, with sensitivity and precision. ■ Next-generation sequencing (NGS) — ddPCR quantifies NGS sample library preparations to increase sequencing accuracy and reduce run repeats. Validate sequencing results such as single nucleotide polymorphisms or copy number variations with absolute quantification. ■ Single cell analysis — the high degree (10-fold to 100-fold) of cell-cell variation in gene expression and genomic content among homogeneous post-mitotic, progenitor, and stem cell populations drives a need for analysis from single cells. ddPCR enables low copy number quantification and gene expression of individual cells. ■ Genome edit detection — ddPCR enables fast, precise, and cost-effective assessment of HDR (Homology directed repair) and NHEJ (non-homologous end joining) generated by CRISPR-Cas9 or other genome editing tools.

Claims	Exemplary Disclosure of Accused Product
	ddPCR has the following benefits for nucleic acid quantification: ■ Unparalleled precision — The massive sample partitioning afforded by ddPCR enables small fold differences in target DNA sequence between samples to be reliably measured. ■ Increased signal-to-noise ratio — High-copy templates and background are diluted, effectively enriching template concentration in target-positive partitions. This allows for the sensitive detection of rare targets and enables a $\pm 10\%$ precision in quantification. ■ Removal of PCR efficiency bias — Error rates are reduced by removing the amplification efficiency reliance of qPCR, enabling accurate quantification of targets to near zero. ■ Simplified quantification — There is no requirement for a standard curve for absolute quantification. 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.

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. PCR Amplification After droplet generation, the plates are moved through the internal thermal cycler, which performs repeated heating and cooling processes. This amplifies one or more copies of a particular DNA segment, thereby multiplying strands of DNA sequencing into the thousands or millions. Droplet Reader Following PCR amplification of the nucleic acid target in the droplets, the plate moves to the Droplet Reader, which analyzes each droplet individually using a four-color detection system, depending on the number of channels specified in the experiment. The autosampler in the Droplet Reader picks up the droplets from each well in the plate. The droplets are spaced out individually for fluorescence reading and fluorescence is then measured in each droplet. Positive droplets, which contain at least one copy of the target DNA molecule, exhibit increased fluorescence compared to negative droplets and are presented in color in the analysis displays. Negative droplets are presented in grayscale. BIO_CC00006554 at 6572-6574. The QX One System can detect up to eight targets using four fluorophores.

Claims	Exemplary Disclosure of Accused Product														
	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. <table><tr><th>ddPCR Supermix</th><th>Description</th></tr><tr><td>Multiplex Supermix</td><td>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.</td></tr><tr><td>Supermix for Probes (No dUTP)</td><td>For use in nucleic acid sample preparations, with sensitivity for the amplification and detection of DNA targets using hydrolysis probe-based assays.</td></tr><tr><td>Supermix for Probes</td><td>For use in sample preparation with uracil N-glycosylase (UNG) decontamination protocols to prevent the reamplification of carryover PCR products between experiments.</td></tr><tr><td>Supermix for Residual DNA Quantification</td><td>For use in residual DNA detection.</td></tr><tr><td>One-Step RT Advanced Kit for Probes</td><td>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.</td></tr><tr><td>EvaGreen® Supermix</td><td>For use in gene expression.</td></tr></table>	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-glycosylase (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.
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Claims	Exemplary Disclosure of Accused Product
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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 <table><tr><th>Assay type</th><th>Description</th></tr><tr><td>Single target per channel</td><td>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.</td></tr><tr><td>Amplitude multiplex</td><td>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.</td></tr><tr><td>Probe mix triplex</td><td>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.</td></tr><tr><td>Advanced classification</td><td>Method to increase multiplexing rate in any combination of targets detected per channel. This method requires manual classification of droplets.</td></tr><tr><td>Basic drop-off</td><td>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.</td></tr></table>	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.
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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.												

Table 10. Assay types, continued

Assay type	Description
Double drop-off	This assay type is available only for the DOF experiment type, and allows two groups of three targets each. The first group appears by default, and looks for one target in FAM <u>and</u> in HEX/VIC (target type reference) one target in FAM (target type unknown), and one target in HEX/VIC (target type unknown). You can add the second group to look for one target in Cy5 <u>and</u> Cy5.5 (target type reference, with two reference copies), one target in Cy5 (target type unknown), and one target in Cy5.5 (target type unknown). By default, all reference copies are set to 2. The channels are static for each group. (Channel 1 FAM is always paired with Channel 2 HEX/VIC, Channel 3 Cy5 is always paired with Channel 4 Cy5.5 .

The experiment type you select determines the available assay methods. [Table 11](#) displays the sample type associated with each assay method.

Table 11. Assay types by experiment type

Assay type	Experiment type						
	DQ	CNV	MUT	DOF	GEX	RDQ	RED
Single target per channel	✓	✓	✓		✓	✓	✓
Amplitude multiplex	✓	✓	✓		✓	✓	✓
Probe mix triplex	✓	✓	✓		✓	✓	✓
Advanced classification	✓						✓
Basic drop-off				✓			
Double drop-off				✓			

QX ONE Software automatically populates the fluorophore in each channel for each target but you can change the default selections.

Use [Table 12](#) to select from the fluorophore options for each assay method listed.

Claims

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Table 12. Fluorophore options

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

BIO_CC00006554 at 6620-6624.

The QX One System uses kits with hybridization probes for the detection of targets, including existing ddPCR Assays for hydrolysis probe chemistry such as TaqMan probe-based assays. A POSA understands that TaqMan probes are oligonucleotide probe attached to a fluorophore and a quencher, commonly known as hybridization probes.

The detection of positive targets corresponds to different colors in the exemplary 2D Amplitude chart below. Each probe corresponds to one of the polynucleotide analytes.

Claims	Exemplary Disclosure of Accused Product
	BIO_CC00006554 at 6652. In Amplitude Multiplex method, the four channels can detect up to eight targets, which will be shown in different color on the 2D Amplitude Graph. The QX One system can be used to perform a probe amplitude multiplex assay that has 8 hybridization probes, with each corresponding to one polynucleotide analyte targets for a total of 8 targets (e.g., four groups of two targets each).
[1a.3] wherein each hybridization probe comprises at least one fluorophore selected from four fluorophores,	The QX One System is used to perform the step of wherein each hybridization probe comprises at least one fluorophore selected from four fluorophores, either literally or under the doctrine of equivalents.

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	Chapter 1 Introduction to Droplet Digital PCR Droplet Digital™ PCR is a digital polymerase chain reaction method based on water-oil emulsion droplet technology. Droplet Digital PCR™ (ddPCR™) uses a combination of microfluidics and proprietary surfactant chemistries to divide each sample into water-in-oil droplets. The technology uses reagents and workflows similar to those used for most standard TaqMan probe-based assays, and provides absolute quantification of nucleic acid target sequences by counting nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions. ddPCR is highly effective in the following areas: ■ Absolute quantification — ddPCR provides a concentration of target DNA copies per input sample without the need for running standard curves, making this technique ideal for target DNA measurements, viral load analysis, and microbial quantification. ■ Genomic alterations such as gene copy number variation (CNV) — CNVs result in too few or too many dosage-sensitive genes responsible for phenotypic variability, complex behavioral traits, and disease. ddPCR enables measurement of 1.2x differences in gene copy number. ■ Detection of rare sequences — researchers must amplify single genes in a complex sample, such as a few tumor cells in a wild-type background. ddPCR is sensitive enough to detect rare mutations or sequences. ■ Gene expression and microRNA analysis — ddPCR provides stand-alone absolute quantification of expression levels, especially low-abundance microRNAs, with sensitivity and precision. ■ Next-generation sequencing (NGS) — ddPCR quantifies NGS sample library preparations to increase sequencing accuracy and reduce run repeats. Validate sequencing results such as single nucleotide polymorphisms or copy number variations with absolute quantification. ■ Single cell analysis — the high degree (10-fold to 100-fold) of cell-cell variation in gene expression and genomic content among homogeneous post-mitotic, progenitor, and stem cell populations drives a need for analysis from single cells. ddPCR enables low copy number quantification and gene expression of individual cells. ■ Genome edit detection — ddPCR enables fast, precise, and cost-effective assessment of HDR (Homology directed repair) and NHEJ (non-homologous end joining) generated by CRISPR-Cas9 or other genome editing tools.

Claims	Exemplary Disclosure of Accused Product
	ddPCR has the following benefits for nucleic acid quantification: ■ Unparalleled precision — The massive sample partitioning afforded by ddPCR enables small fold differences in target DNA sequence between samples to be reliably measured. ■ Increased signal-to-noise ratio — High-copy templates and background are diluted, effectively enriching template concentration in target-positive partitions. This allows for the sensitive detection of rare targets and enables a $\pm 10\%$ precision in quantification. ■ Removal of PCR efficiency bias — Error rates are reduced by removing the amplification efficiency reliance of qPCR, enabling accurate quantification of targets to near zero. ■ Simplified quantification — There is no requirement for a standard curve for absolute quantification. 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.

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	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. PCR Amplification After droplet generation, the plates are moved through the internal thermal cycler, which performs repeated heating and cooling processes. This amplifies one or more copies of a particular DNA segment, thereby multiplying strands of DNA sequencing into the thousands or millions. Droplet Reader Following PCR amplification of the nucleic acid target in the droplets, the plate moves to the Droplet Reader, which analyzes each droplet individually using a four-color detection system, depending on the number of channels specified in the experiment. The autosampler in the Droplet Reader picks up the droplets from each well in the plate. The droplets are spaced out individually for fluorescence reading and fluorescence is then measured in each droplet. Positive droplets, which contain at least one copy of the target DNA molecule, exhibit increased fluorescence compared to negative droplets and are presented in color in the analysis displays. Negative droplets are presented in grayscale. BIO_CC00006554 at 6572-6574. The QX One System can detect up to eight targets using four fluorophores.

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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.												

Table 10. Assay types, continued

Assay type	Description
Double drop-off	This assay type is available only for the DOF experiment type, and allows two groups of three targets each. The first group appears by default, and looks for one target in FAM <u>and</u> in HEX/VIC (target type reference) one target in FAM (target type unknown), and one target in HEX/VIC (target type unknown). You can add the second group to look for one target in Cy5 <u>and</u> Cy5.5 (target type reference, with two reference copies), one target in Cy5 (target type unknown), and one target in Cy5.5 (target type unknown). By default, all reference copies are set to 2. The channels are static for each group. (Channel 1 FAM is always paired with Channel 2 HEX/VIC, Channel 3 Cy5 is always paired with Channel 4 Cy5.5 .

The experiment type you select determines the available assay methods. [Table 11](#) displays the sample type associated with each assay method.

Table 11. Assay types by experiment type

Assay type	Experiment type						
	DQ	CNV	MUT	DOF	GEX	RDQ	RED
Single target per channel	✓	✓	✓		✓	✓	✓
Amplitude multiplex	✓	✓	✓		✓	✓	✓
Probe mix triplex	✓	✓	✓		✓	✓	✓
Advanced classification	✓						✓
Basic drop-off				✓			
Double drop-off				✓			

QX ONE Software automatically populates the fluorophore in each channel for each target but you can change the default selections.

Use [Table 12](#) to select from the fluorophore options for each assay method listed.

Claims

Exemplary Disclosure of Accused Product

Table 12. Fluorophore options

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

BIO_CC00006554 at 6620-6624. As shown above, at least four fluorophores (FAM, HEX/VIC, Cy5, and Cy5.5) are detectable and each hybridization probe corresponds to a specific fluorophore or a combination of fluorophores. In the above table, for the Amplitude multiplex Assay, the QX One system is used to provide each hybridization probe (such as TaqMan probes) that comprises at least one fluorophore selected from four fluorophores. As described in the table above, each of the probes corresponds to one of the polynucleotide analytes targets.

Claims	Exemplary Disclosure of Accused Product
	FEATURES AND BENEFITS  WALK-AWAY OPERATION ▪ The QX ONE System automates a fully integrated ddPCR workflow, allowing for walk-away operation ▪ A 5-plate capacity allows 480 samples to be analyzed in a single day  EASY TO USE ▪ Simple, intuitive run setup using a large onboard touch screen ▪ Optimized plate setup means fewer handling steps, fewer operator errors, and faster onboarding  ADVANCED MULTIPLEXING ▪ Four color channels combined with amplitude multiplexing and/or radial multiplexing allow for multiple unique targets to be detected in a single reaction ▪ Specially formulated ddPCR Multiplex Supermix is optimized to empower advanced multiplexing in probe-based assays  REGULATORY COMPLIANT ▪ QX ONE Software, Regulatory Edition is a robust package that enables U.S. FDA 21 CFR Part 11 compliance and offers audit trails with tracked protocol changes for regulatory requirements ▪ RFID barcoded consumables allow lot management and traceability BIO_CC00008515 at 8517.

Claims	Exemplary Disclosure of Accused Product
[1a.4] and wherein each hybridization probe, when excited and when contacted with its corresponding polynucleotide analyte, generates a cumulative intensity signal,	The QX One System is used to perform the step of wherein each hybridization probe, when excited and when contacted with its corresponding polynucleotide analyte, generates a cumulative intensity signal either literally or under the doctrine of equivalents. For example, The QX ONE uses reagents and workflows similar to those used for most standard TaqMan probe-based assays: Chapter 1 Introduction to Droplet Digital PCR Droplet Digital™ PCR is a digital polymerase chain reaction method based on water-oil emulsion droplet technology. Droplet Digital PCR™ (ddPCR™) uses a combination of microfluidics and proprietary surfactant chemistries to divide each sample into water-in-oil droplets. The technology uses reagents and workflows similar to those used for most standard TaqMan probe-based assays, and provides absolute quantification of nucleic acid target sequences by counting nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions. ddPCR is highly effective in the following areas: ■ Absolute quantification — ddPCR provides a concentration of target DNA copies per input sample without the need for running standard curves, making this technique ideal for target DNA measurements, viral load analysis, and microbial quantification. ■ Genomic alterations such as gene copy number variation (CNV) — CNVs result in too few or too many dosage-sensitive genes responsible for phenotypic variability, complex behavioral traits, and disease. ddPCR enables measurement of 1.2x differences in gene copy number. ■ Detection of rare sequences — researchers must amplify single genes in a complex sample, such as a few tumor cells in a wild-type background. ddPCR is sensitive enough to detect rare mutations or sequences. ■ Gene expression and microRNA analysis — ddPCR provides stand-alone absolute quantification of expression levels, especially low-abundance microRNAs, with sensitivity and precision. ■ Next-generation sequencing (NGS) — ddPCR quantifies NGS sample library preparations to increase sequencing accuracy and reduce run repeats. Validate sequencing results such as single nucleotide polymorphisms or copy number variations with absolute quantification. ■ Single cell analysis — the high degree (10-fold to 100-fold) of cell-cell variation in gene expression and genomic content among homogeneous post-mitotic, progenitor, and stem cell populations drives a need for analysis from single cells. ddPCR enables low copy number quantification and gene expression of individual cells. ■ Genome edit detection — ddPCR enables fast, precise, and cost-effective assessment of HDR (Homology directed repair) and NHEJ (non-homologous end joining) generated by CRISPR-Cas9 or other genome editing tools.

Claims	Exemplary Disclosure of Accused Product
	ddPCR has the following benefits for nucleic acid quantification: ■ Unparalleled precision — The massive sample partitioning afforded by ddPCR enables small fold differences in target DNA sequence between samples to be reliably measured. ■ Increased signal-to-noise ratio — High-copy templates and background are diluted, effectively enriching template concentration in target-positive partitions. This allows for the sensitive detection of rare targets and enables a $\pm 10\%$ precision in quantification. ■ Removal of PCR efficiency bias — Error rates are reduced by removing the amplification efficiency reliance of qPCR, enabling accurate quantification of targets to near zero. ■ Simplified quantification — There is no requirement for a standard curve for absolute quantification. 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.

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. PCR Amplification After droplet generation, the plates are moved through the internal thermal cycler, which performs repeated heating and cooling processes. This amplifies one or more copies of a particular DNA segment, thereby multiplying strands of DNA sequencing into the thousands or millions. Droplet Reader Following PCR amplification of the nucleic acid target in the droplets, the plate moves to the Droplet Reader, which analyzes each droplet individually using a four-color detection system, depending on the number of channels specified in the experiment. The autosampler in the Droplet Reader picks up the droplets from each well in the plate. The droplets are spaced out individually for fluorescence reading and fluorescence is then measured in each droplet. Positive droplets, which contain at least one copy of the target DNA molecule, exhibit increased fluorescence compared to negative droplets and are presented in color in the analysis displays. Negative droplets are presented in grayscale. BIO_CCODE00006554 at 6572-6574. Under the process used by the QX ONE, the probe containing the fluorophores is mixed into the sample, e.g., during sample preparation/provision as described above. The sample then undergoes a thermal cycling as described above. Thereafter, the QX ONE excites the fluorophores on the probes in the sample using, for example and on information and belief, light at different wavelengths. This results in the hybridization probe (and the fluorophore component thereof) becoming excited. If the polynucleotide analyte is in contact with the hybridization probe, a cumulative intensity signal is generated. An exemplary process for sample preparation wherein the hybridization probe is excited placed into contact with its corresponding polynucleotide analyte is describe at https://www.bio-

Claims	Exemplary Disclosure of Accused Product														
	rad.com/webroot/web/pdf/lsr/literature/Bulletin_6407.pdf at 4-5, 12-13, 15-17. On information and belief, the same or materially similar process is followed for preparing samples for the QX ONE. See e.g., BIO_CC00007875, BIO_CC00007877, BIO_CC00007879, and BIO_CC00007881. The result is that the sample exhibits varying degrees of dye fluorescence which represent the cumulative intensity signals, as discussed above. 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. <table> <tr> <th>ddPCR Supermix</th><th>Description</th></tr> <tr> <td>Multiplex Supermix</td><td>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.</td></tr> <tr> <td>Supermix for Probes (No dUTP)</td><td>For use in nucleic acid sample preparations, with sensitivity for the amplification and detection of DNA targets using hydrolysis probe-based assays.</td></tr> <tr> <td>Supermix for Probes</td><td>For use in sample preparation with uracil N-glycosylase (UNG) decontamination protocols to prevent the reamplification of carryover PCR products between experiments.</td></tr> <tr> <td>Supermix for Residual DNA Quantification</td><td>For use in residual DNA detection.</td></tr> <tr> <td>One-Step RT Advanced Kit for Probes</td><td>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.</td></tr> <tr> <td>EvaGreen® Supermix</td><td>For use in gene expression.</td></tr> </table>	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-glycosylase (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.
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-glycosylase (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.														

Claims	Exemplary Disclosure of Accused Product
	Using the One Step RT ddPCR Advanced Kit for Probes The One Step RT ddPCR Advance Kit for Probes supermix is time and temperature-sensitive. Therefore, if you are performing an experiment using this supermix, it is important that the plate does not sit idle in one of the Inbox slots. To perform an experiment using this kit 1. Wait until all plates in the QX ONE Droplet Digital PCR System have processed and then place the One Step RT plate into the first slot. 2. Quickly set up and start the run. For information, see Plate Configuration Window on page 56 and Running Experiments on page 71 Running Experiments on page 71.

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 <table><tr><th>Assay type</th><th>Description</th></tr><tr><td>Single target per channel</td><td>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.</td></tr><tr><td>Amplitude multiplex</td><td>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.</td></tr><tr><td>Probe mix triplex</td><td>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.</td></tr><tr><td>Advanced classification</td><td>Method to increase multiplexing rate in any combination of targets detected per channel. This method requires manual classification of droplets.</td></tr><tr><td>Basic drop-off</td><td>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.</td></tr></table>	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.
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.												
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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.												

Table 10. Assay types, continued

Assay type	Description
Double drop-off	This assay type is available only for the DOF experiment type, and allows two groups of three targets each. The first group appears by default, and looks for one target in FAM <u>and</u> in HEX/VIC (target type reference) one target in FAM (target type unknown), and one target in HEX/VIC (target type unknown). You can add the second group to look for one target in Cy5 <u>and</u> Cy5.5 (target type reference, with two reference copies), one target in Cy5 (target type unknown), and one target in Cy5.5 (target type unknown). By default, all reference copies are set to 2. The channels are static for each group. (Channel 1 FAM is always paired with Channel 2 HEX/VIC, Channel 3 Cy5 is always paired with Channel 4 Cy5.5 .

The experiment type you select determines the available assay methods. [Table 11](#) displays the sample type associated with each assay method.

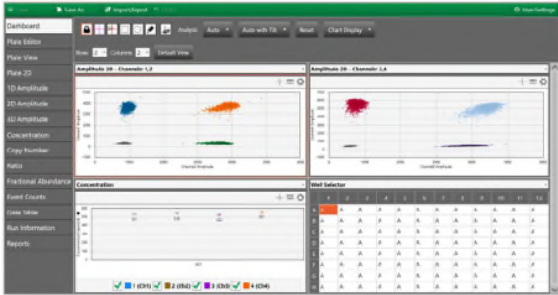
Table 11. Assay types by experiment type

Assay type	Experiment type						
	DQ	CNV	MUT	DOF	GEX	RDQ	RED
Single target per channel	✓	✓	✓		✓	✓	✓
Amplitude multiplex	✓	✓	✓		✓	✓	✓
Probe mix triplex	✓	✓	✓		✓	✓	✓
Advanced classification	✓						✓
Basic drop-off				✓			
Double drop-off				✓			

QX ONE Software automatically populates the fluorophore in each channel for each target but you can change the default selections.

Use [Table 12](#) to select from the fluorophore options for each assay method listed.

Claims	Exemplary Disclosure of Accused Product																																																																									
	Table 12. Fluorophore options <table><tr><th>Assay method</th><th>Signal Ch1</th><th>Signal Ch2</th><th>Signal Ch3</th><th>Signal Ch4</th></tr><tr><td rowspan="3">Single target per channel, for 1 to 4 targets</td><td>FAM</td><td>HEX</td><td>Cy5</td><td>Cy5.5</td></tr><tr><td>None</td><td>VIC</td><td>None</td><td>None</td></tr><tr><td></td><td>None</td><td></td><td></td></tr><tr><td rowspan="5">Amplitude multiplex, for 1 to 8 targets</td><td>FAM Hi</td><td>HEX Hi</td><td>Cy5 Hi</td><td>Cy5.5 Hi</td></tr><tr><td>FAM Lo</td><td>HEX Lo</td><td>Cy 5 Lo</td><td>Cy 5.5 Lo</td></tr><tr><td>None</td><td>VIC Hi</td><td>None</td><td>None</td></tr><tr><td></td><td>VIC Lo</td><td></td><td></td></tr><tr><td></td><td>None</td><td></td><td></td></tr><tr><td rowspan="3">Probe mix triplex, for 6 targets</td><td>FAM</td><td>HEX</td><td>Cy5</td><td>Cy5.5</td></tr><tr><td>None</td><td>VIC</td><td>None</td><td>None</td></tr><tr><td></td><td>None</td><td></td><td></td></tr><tr><td rowspan="5">Advanced classification</td><td>FAM</td><td>HEX</td><td>Cy5</td><td>Cy5.5</td></tr><tr><td>+1-10</td><td>1 to 10</td><td>1 to 10</td><td>1 to 10</td></tr><tr><td>None</td><td>VIC</td><td>None</td><td>None</td></tr><tr><td></td><td>1 to 10</td><td></td><td></td></tr><tr><td></td><td>None</td><td></td><td></td></tr></table> BIO_CC00006554 at 6620-6624. FEATURES AND BENEFITS  WALK-AWAY OPERATION - The QX ONE System automates a fully integrated ddPCR workflow, allowing for walk-away operation - A 5-plate capacity allows 480 samples to be analyzed in a single day  EASY TO USE - Simple, intuitive run setup using a large onboard touch screen - Optimized plate setup means fewer handling steps, fewer operator errors, and faster onboarding  ADVANCED MULTIPLEXING - Four color channels combined with amplitude multiplexing and/or radial multiplexing allow for multiple unique targets to be detected in a single reaction - Specialty formulated ddPCR Multiplex Supermix is optimized to empower advanced multiplexing in probe-based assays  REGULATORY COMPLIANT - QX ONE Software, Regulatory Edition is a robust package that enables U.S. FDA 21 CFR Part 11 compliance and offers audit trails with tracked protocol changes for regulatory requirements - RFID barcoded consumables allow lot management and traceability BIO_CC00008489 at 8490.	Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4	Single target per channel, for 1 to 4 targets	FAM	HEX	Cy5	Cy5.5	None	VIC	None	None		None			Amplitude multiplex, for 1 to 8 targets	FAM Hi	HEX Hi	Cy5 Hi	Cy5.5 Hi	FAM Lo	HEX Lo	Cy 5 Lo	Cy 5.5 Lo	None	VIC Hi	None	None		VIC Lo				None			Probe mix triplex, for 6 targets	FAM	HEX	Cy5	Cy5.5	None	VIC	None	None		None			Advanced classification	FAM	HEX	Cy5	Cy5.5	+1-10	1 to 10	1 to 10	1 to 10	None	VIC	None	None		1 to 10				None		
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Claims	Exemplary Disclosure of Accused Product
	Analysis Dashboard When you open a data file, the Analysis module opens to a Dashboard window containing default analysis and data panes in a summary view. You can customize the view to change panes, or increase or decrease the number of panes. For information on the available options in the Dashboard view, see Dashboard Options on page 84. For more information on the Analysis Dashboard, refer to the QX ONE Software User Guide.  Note: If you open a .qlo file, the Plate Editor opens as the default view and you must select the Dashboard tab to open it. From the tabs on the left, you can access plate views, amplitude charts, data visualization distribution charts, droplet counts, and a complete data table. You can also view data specific to the run in the Run Information window, and run reports on any of the analysis charts from the Reports window. BIO_CC00006554 at 6652-6654.
[1a.5] further wherein each generated cumulative intensity signal comprises at least one signal generated by excitement of at	Plaintiffs incorporate their discussion from [1a.4] above here. When the hybridization probes are excited in accordance with performing step 1a.4 above, the fluorophore becomes amplified/excited by the same process. See https://www.biorad.com/webroot/web/pdf/lsr/literature/Bulletin_6407.pdf at 4-5, 12-13. On information and belief, the same or materially similar process is followed for preparing samples for the QX ONE. See also:

Claims	Exemplary Disclosure of Accused Product
least one of the four fluorophores;	Chapter 1 Introduction to Droplet Digital PCR Droplet Digital™ PCR is a digital polymerase chain reaction method based on water-oil emulsion droplet technology. Droplet Digital PCR™ (ddPCR™) uses a combination of microfluidics and proprietary surfactant chemistries to divide each sample into water-in-oil droplets. The technology uses reagents and workflows similar to those used for most standard TaqMan probe-based assays, and provides absolute quantification of nucleic acid target sequences by counting nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions. ddPCR is highly effective in the following areas: ■ Absolute quantification — ddPCR provides a concentration of target DNA copies per input sample without the need for running standard curves, making this technique ideal for target DNA measurements, viral load analysis, and microbial quantification. ■ Genomic alterations such as gene copy number variation (CNV) — CNVs result in too few or too many dosage-sensitive genes responsible for phenotypic variability, complex behavioral traits, and disease. ddPCR enables measurement of 1.2x differences in gene copy number. ■ Detection of rare sequences — researchers must amplify single genes in a complex sample, such as a few tumor cells in a wild-type background. ddPCR is sensitive enough to detect rare mutations or sequences. ■ Gene expression and microRNA analysis — ddPCR provides stand-alone absolute quantification of expression levels, especially low-abundance microRNAs, with sensitivity and precision. ■ Next-generation sequencing (NGS) — ddPCR quantifies NGS sample library preparations to increase sequencing accuracy and reduce run repeats. Validate sequencing results such as single nucleotide polymorphisms or copy number variations with absolute quantification. ■ Single cell analysis — the high degree (10-fold to 100-fold) of cell-cell variation in gene expression and genomic content among homogeneous post-mitotic, progenitor, and stem cell populations drives a need for analysis from single cells. ddPCR enables low copy number quantification and gene expression of individual cells. ■ Genome edit detection — ddPCR enables fast, precise, and cost-effective assessment of HDR (Homology directed repair) and NHEJ (non-homologous end joining) generated by CRISPR-Cas9 or other genome editing tools.

Claims	Exemplary Disclosure of Accused Product
	ddPCR has the following benefits for nucleic acid quantification: ■ Unparalleled precision — The massive sample partitioning afforded by ddPCR enables small fold differences in target DNA sequence between samples to be reliably measured. ■ Increased signal-to-noise ratio — High-copy templates and background are diluted, effectively enriching template concentration in target-positive partitions. This allows for the sensitive detection of rare targets and enables a $\pm 10\%$ precision in quantification. ■ Removal of PCR efficiency bias — Error rates are reduced by removing the amplification efficiency reliance of qPCR, enabling accurate quantification of targets to near zero. ■ Simplified quantification — There is no requirement for a standard curve for absolute quantification. 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.

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. PCR Amplification After droplet generation, the plates are moved through the internal thermal cycler, which performs repeated heating and cooling processes. This amplifies one or more copies of a particular DNA segment, thereby multiplying strands of DNA sequencing into the thousands or millions. Droplet Reader Following PCR amplification of the nucleic acid target in the droplets, the plate moves to the Droplet Reader, which analyzes each droplet individually using a four-color detection system, depending on the number of channels specified in the experiment. The autosampler in the Droplet Reader picks up the droplets from each well in the plate. The droplets are spaced out individually for fluorescence reading and fluorescence is then measured in each droplet. Positive droplets, which contain at least one copy of the target DNA molecule, exhibit increased fluorescence compared to negative droplets and are presented in color in the analysis displays. Negative droplets are presented in grayscale. BIO_CCODE00006554 at 6572-6574.

Claims	Exemplary Disclosure of Accused Product														
	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. <table><tr><th>ddPCR Supermix</th><th>Description</th></tr><tr><td>Multiplex Supermix</td><td>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.</td></tr><tr><td>Supermix for Probes (No dUTP)</td><td>For use in nucleic acid sample preparations, with sensitivity for the amplification and detection of DNA targets using hydrolysis probe-based assays.</td></tr><tr><td>Supermix for Probes</td><td>For use in sample preparation with uracil N-glycosylase (UNG) decontamination protocols to prevent the reamplification of carryover PCR products between experiments.</td></tr><tr><td>Supermix for Residual DNA Quantification</td><td>For use in residual DNA detection.</td></tr><tr><td>One-Step RT Advanced Kit for Probes</td><td>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.</td></tr><tr><td>EvaGreen® Supermix</td><td>For use in gene expression.</td></tr></table>	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-glycosylase (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.
ddPCR Supermix	Description														
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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.														

Claims	Exemplary Disclosure of Accused Product
	Using the One Step RT ddPCR Advanced Kit for Probes The One Step RT ddPCR Advance Kit for Probes supermix is time and temperature-sensitive. Therefore, if you are performing an experiment using this supermix, it is important that the plate does not sit idle in one of the Inbox slots. To perform an experiment using this kit 1. Wait until all plates in the QX ONE Droplet Digital PCR System have processed and then place the One Step RT plate into the first slot. 2. Quickly set up and start the run. For information, see Plate Configuration Window on page 56 and Running Experiments on page 71 Running Experiments on page 71.

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 <table><tr><th>Assay type</th><th>Description</th></tr><tr><td>Single target per channel</td><td>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.</td></tr><tr><td>Amplitude multiplex</td><td>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.</td></tr><tr><td>Probe mix triplex</td><td>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.</td></tr><tr><td>Advanced classification</td><td>Method to increase multiplexing rate in any combination of targets detected per channel. This method requires manual classification of droplets.</td></tr><tr><td>Basic drop-off</td><td>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.</td></tr></table>	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.
Assay type	Description												
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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.												

Table 10. Assay types, continued

Assay type	Description
Double drop-off	This assay type is available only for the DOF experiment type, and allows two groups of three targets each. The first group appears by default, and looks for one target in FAM <u>and</u> in HEX/VIC (target type reference) one target in FAM (target type unknown), and one target in HEX/VIC (target type unknown). You can add the second group to look for one target in Cy5 <u>and</u> Cy5.5 (target type reference, with two reference copies), one target in Cy5 (target type unknown), and one target in Cy5.5 (target type unknown). By default, all reference copies are set to 2. The channels are static for each group. (Channel 1 FAM is always paired with Channel 2 HEX/VIC, Channel 3 Cy5 is always paired with Channel 4 Cy5.5 .

The experiment type you select determines the available assay methods. [Table 11](#) displays the sample type associated with each assay method.

Table 11. Assay types by experiment type

Assay type	Experiment type						
	DQ	CNV	MUT	DOF	GEX	RDQ	RED
Single target per channel	✓	✓	✓		✓	✓	✓
Amplitude multiplex	✓	✓	✓		✓	✓	✓
Probe mix triplex	✓	✓	✓		✓	✓	✓
Advanced classification	✓						✓
Basic drop-off				✓			
Double drop-off				✓			

QX ONE Software automatically populates the fluorophore in each channel for each target but you can change the default selections.

Use [Table 12](#) to select from the fluorophore options for each assay method listed.

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Table 12. Fluorophore options

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

BIO_CCODE00006554 at 6620-6624.

Default color assignments for 2D amplitude plots are:

Positive FAM	=	Blue
Positive HEX/VIC	=	Green
Positive FAM and HEX/VIC	=	Orange (may appear brown on some monitors)
Positive Cy5	=	Red
Positive Cy5.5	=	Violet
Positive Cy5 and Cy5.5	=	Light Blue
Double negative	=	Gray

QX ONE Software Standard Edition User Guide Version 1.4, BIO_ BIO_CCODE00007041 at 7158.

Claims	Exemplary Disclosure of Accused Product
[1b] b) contacting said sample solution volume with the plurality of hybridization probes and exciting the hybridization probes to generate the cumulative intensity signal(s) if one or more of the at least seven polynucleotide analytes is present in the sample volume; and	Plaintiffs incorporate by reference the discussion from Steps 1a.4 and 1a.5. When the process described above is performed using a QX ONE, the sample solution described above in step 1a will be contacted with the various hybridization probes and excited through the process described in Steps 1a.4 and 1a.5. The cumulative intensity signals are generated in accordance with Step 1a.4 and 1a.5 based on the hybridization probes contacting the corresponding polynucleotide. The “at least seven” limitation occurs when contact is made between the hybridization probes and at least seven corresponding polynucleotides in accordance with the description in Step 1a. The QX One System is used to perform the step of contacting said sample solution volume with the plurality of hybridization probes either literally or under the doctrine of equivalents.
[1c.1] c) measuring the cumulative intensity signal(s)	The QX One System is used to perform measuring the cumulative intensity signals either literally or under the doctrine of equivalents. The QX One is used to measure the cumulative intensity signals.

Claims	Exemplary Disclosure of Accused Product
	Chapter 1 Introduction to Droplet Digital PCR Droplet Digital™ PCR is a digital polymerase chain reaction method based on water-oil emulsion droplet technology. Droplet Digital PCR™ (ddPCR™) uses a combination of microfluidics and proprietary surfactant chemistries to divide each sample into water-in-oil droplets. The technology uses reagents and workflows similar to those used for most standard TaqMan probe-based assays, and provides absolute quantification of nucleic acid target sequences by counting nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions. ddPCR is highly effective in the following areas: ■ Absolute quantification — ddPCR provides a concentration of target DNA copies per input sample without the need for running standard curves, making this technique ideal for target DNA measurements, viral load analysis, and microbial quantification. ■ Genomic alterations such as gene copy number variation (CNV) — CNVs result in too few or too many dosage-sensitive genes responsible for phenotypic variability, complex behavioral traits, and disease. ddPCR enables measurement of 1.2x differences in gene copy number. ■ Detection of rare sequences — researchers must amplify single genes in a complex sample, such as a few tumor cells in a wild-type background. ddPCR is sensitive enough to detect rare mutations or sequences. ■ Gene expression and microRNA analysis — ddPCR provides stand-alone absolute quantification of expression levels, especially low-abundance microRNAs, with sensitivity and precision. ■ Next-generation sequencing (NGS) — ddPCR quantifies NGS sample library preparations to increase sequencing accuracy and reduce run repeats. Validate sequencing results such as single nucleotide polymorphisms or copy number variations with absolute quantification. ■ Single cell analysis — the high degree (10-fold to 100-fold) of cell-cell variation in gene expression and genomic content among homogeneous post-mitotic, progenitor, and stem cell populations drives a need for analysis from single cells. ddPCR enables low copy number quantification and gene expression of individual cells. ■ Genome edit detection — ddPCR enables fast, precise, and cost-effective assessment of HDR (Homology directed repair) and NHEJ (non-homologous end joining) generated by CRISPR-Cas9 or other genome editing tools.

Claims	Exemplary Disclosure of Accused Product
	ddPCR has the following benefits for nucleic acid quantification: ■ Unparalleled precision — The massive sample partitioning afforded by ddPCR enables small fold differences in target DNA sequence between samples to be reliably measured. ■ Increased signal-to-noise ratio — High-copy templates and background are diluted, effectively enriching template concentration in target-positive partitions. This allows for the sensitive detection of rare targets and enables a $\pm 10\%$ precision in quantification. ■ Removal of PCR efficiency bias — Error rates are reduced by removing the amplification efficiency reliance of qPCR, enabling accurate quantification of targets to near zero. ■ Simplified quantification — There is no requirement for a standard curve for absolute quantification. 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.

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. PCR Amplification After droplet generation, the plates are moved through the internal thermal cycler, which performs repeated heating and cooling processes. This amplifies one or more copies of a particular DNA segment, thereby multiplying strands of DNA sequencing into the thousands or millions. Droplet Reader Following PCR amplification of the nucleic acid target in the droplets, the plate moves to the Droplet Reader, which analyzes each droplet individually using a four-color detection system, depending on the number of channels specified in the experiment. The autosampler in the Droplet Reader picks up the droplets from each well in the plate. The droplets are spaced out individually for fluorescence reading and fluorescence is then measured in each droplet. Positive droplets, which contain at least one copy of the target DNA molecule, exhibit increased fluorescence compared to negative droplets and are presented in color in the analysis displays. Negative droplets are presented in grayscale. BIO_CC00006554 at 6572-6574. The QX ONE system provides ways of measuring the cumulative intensity signals, for example, signal from the hydrolysis probes is generated, for example, when they become attached to the analytes targets and is generated by means of an extension reaction by common hydrolysis probe chemistry in using hydrolysis probe-based assays.

Claims	Exemplary Disclosure of Accused Product																
	Experiment Types QX ONE Software offers the experiment types described in Table 9, each enabling different assay options and analysis tools. You can assign multiple experiment types across the plate, but you can assign only one experiment type to each well. Table 9. Experiment types <table><tr><th>Type</th><th>Description</th></tr><tr><td>Direct quantification (DQ)</td><td>DQ uses absolute quantification to determine the concentration (copies/ul) of target DNA copies in a sample. DQ measures the number of positive and negative droplets for each target in a well based on their fluorescence amplitude, and uses a Poisson algorithm to calculate the starting concentration of each target DNA molecule. Note: The ABS experiment type created in QuantaSoft Software versions 1.4 through 1.7 is automatically mapped into the software as a DQ experiment type.</td></tr><tr><td>Copy number variation (CNV)</td><td>CNV determines concentration (as with DQ) and calculates the copy number of an unknown (CNV) target relative to a known reference or references within the same well.</td></tr><tr><td>Mutation detection (MUT)</td><td>MUT determines concentration (as with DQ) and calculates the fractional abundance of an unknown mutant present at a low frequency in a wild-type background.</td></tr><tr><td>Rare event detection (RED)</td><td>RED determines the concentration of a known mutant or a rare target species relative to a given reference species within a large pool of background DNA.</td></tr><tr><td>Drop-off (DOF)</td><td>DOF determines absolute quantification of targets for assays designed to detect non-wild type sequences, such as indels and genome edits. DOF is designed to support an assay strategy where one probe counts all alleles and one 'drop-off' probe sits on top of a predicted cut site or mutation site.</td></tr><tr><td>Gene expression (GEX)</td><td>GEX determines concentration (as with DQ) and calculates relative expression levels of an unknown target relative to a known reference or references within the same well.</td></tr><tr><td>Residual DNA quantification (RDQ)</td><td>RDQ provides a precise method for residual host cell DNA quantification. Note: Users can enter a conversion factor to calculate the mass for a well, up to three decimals. The software displays the result in the Molecular Weight column of the Data Table.</td></tr></table>	Type	Description	Direct quantification (DQ)	DQ uses absolute quantification to determine the concentration (copies/ul) of target DNA copies in a sample. DQ measures the number of positive and negative droplets for each target in a well based on their fluorescence amplitude, and uses a Poisson algorithm to calculate the starting concentration of each target DNA molecule. Note: The ABS experiment type created in QuantaSoft Software versions 1.4 through 1.7 is automatically mapped into the software as a DQ experiment type.	Copy number variation (CNV)	CNV determines concentration (as with DQ) and calculates the copy number of an unknown (CNV) target relative to a known reference or references within the same well.	Mutation detection (MUT)	MUT determines concentration (as with DQ) and calculates the fractional abundance of an unknown mutant present at a low frequency in a wild-type background.	Rare event detection (RED)	RED determines the concentration of a known mutant or a rare target species relative to a given reference species within a large pool of background DNA.	Drop-off (DOF)	DOF determines absolute quantification of targets for assays designed to detect non-wild type sequences, such as indels and genome edits. DOF is designed to support an assay strategy where one probe counts all alleles and one 'drop-off' probe sits on top of a predicted cut site or mutation site.	Gene expression (GEX)	GEX determines concentration (as with DQ) and calculates relative expression levels of an unknown target relative to a known reference or references within the same well.	Residual DNA quantification (RDQ)	RDQ provides a precise method for residual host cell DNA quantification. Note: Users can enter a conversion factor to calculate the mass for a well, up to three decimals. The software displays the result in the Molecular Weight column of the Data Table.
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EvaGreen® Supermix	For use in gene expression.														

Claims	Exemplary Disclosure of Accused Product
	Using the One Step RT ddPCR Advanced Kit for Probes The One Step RT ddPCR Advance Kit for Probes supermix is time and temperature-sensitive. Therefore, if you are performing an experiment using this supermix, it is important that the plate does not sit idle in one of the Inbox slots. To perform an experiment using this kit 1. Wait until all plates in the QX ONE Droplet Digital PCR System have processed and then place the One Step RT plate into the first slot. 2. Quickly set up and start the run. For information, see Plate Configuration Window on page 56 and Running Experiments on page 71 Running Experiments on page 71.

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 <table><tr><th>Assay type</th><th>Description</th></tr><tr><td>Single target per channel</td><td>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.</td></tr><tr><td>Amplitude multiplex</td><td>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.</td></tr><tr><td>Probe mix triplex</td><td>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.</td></tr><tr><td>Advanced classification</td><td>Method to increase multiplexing rate in any combination of targets detected per channel. This method requires manual classification of droplets.</td></tr><tr><td>Basic drop-off</td><td>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.</td></tr></table>	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.
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.												

Table 10. Assay types, continued

Assay type	Description
Double drop-off	This assay type is available only for the DOF experiment type, and allows two groups of three targets each. The first group appears by default, and looks for one target in FAM <u>and</u> in HEX/VIC (target type reference) one target in FAM (target type unknown), and one target in HEX/VIC (target type unknown). You can add the second group to look for one target in Cy5 <u>and</u> Cy5.5 (target type reference, with two reference copies), one target in Cy5 (target type unknown), and one target in Cy5.5 (target type unknown). By default, all reference copies are set to 2. The channels are static for each group. (Channel 1 FAM is always paired with Channel 2 HEX/VIC, Channel 3 Cy5 is always paired with Channel 4 Cy5.5 .

The experiment type you select determines the available assay methods. [Table 11](#) displays the sample type associated with each assay method.

Table 11. Assay types by experiment type

Assay type	Experiment type						
	DQ	CNV	MUT	DOF	GEX	RDQ	RED
Single target per channel	✓	✓	✓		✓	✓	✓
Amplitude multiplex	✓	✓	✓		✓	✓	✓
Probe mix triplex	✓	✓	✓		✓	✓	✓
Advanced classification	✓						✓
Basic drop-off				✓			
Double drop-off				✓			

QX ONE Software automatically populates the fluorophore in each channel for each target but you can change the default selections.

Use [Table 12](#) to select from the fluorophore options for each assay method listed.

Claims

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Table 12. Fluorophore options

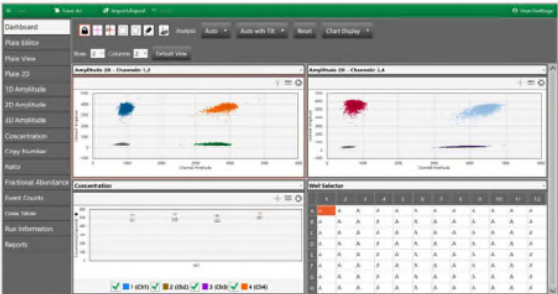
Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4
Single target per channel, for 1 to 4 targets	FAM	HEX	Cy5	Cy5.5
	None	VIC	None	None
		None		
Amplitude multiplex, for 1 to 8 targets	FAM Hi	HEX Hi	Cy5 Hi	Cy5.5 Hi
	FAM Lo	HEX Lo	Cy 5 Lo	Cy 5.5 Lo
	None	VIC Hi	None	None
		VIC Lo		
		None		
Probe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

BIO_CC00006554 at 6620-6624.

The Analysis Dashboard shows the result of measuring cumulative intensity for each fluorophore.

Analysis Dashboard

When you open a data file, the Analysis module opens to a Dashboard window containing default analysis and data panes in a summary view. You can customize the view to change panes, or increase or decrease the number of panes. For information on the available options in the Dashboard view, see [Dashboard Options on page 84](#). For more information on the Analysis Dashboard, refer to the QX ONE Software User Guide.



Note: If you open a .qlo file, the Plate Editor opens as the default view and you must select the Dashboard tab to open it.

From the tabs on the left, you can access plate views, amplitude charts, data visualization distribution charts, droplet counts, and a complete data table. You can also view data specific to the run in the Run Information window, and run reports on any of the analysis charts from the Reports window.

Dashboard Options

From the Dashboard window, depending on the individual dashboard window you have selected, you can use a range of options as described below:

- When you select a window, the options that appear in the menu bar correspond to the options you see if you open the same window from the side menu. For example, if you select the Concentration chart in the Dashboard, the Concentration menu options appear.



Claims	Exemplary Disclosure of Accused Product
	Table 15 describes the option sets identified in the following graphic.  BIO_CC00006554 at 6652-6654. The QX ONE system is used to perform different experiment and assay types to measure the cumulative intensity signals, such as amplitude multiplex and probe mix triplex experiments and assays.
[1c.2] thereby generating a cumulative signal measurement, wherein the cumulative signal measurement non-degenerately	The QX One System is used to generate a cumulative signal measurement, wherein the cumulative signal measurement non-degenerately indicates the presence or absence of the at least seven polynucleotide analytes, in any combination of presence or absence of the at least seven polynucleotide analytes, in any combination of presence or absence, either literally or under the doctrine of equivalents The signals non-degenerately indicates the presence or absence of the targeted analytes, based on the experiment types.

Claims	Exemplary Disclosure of Accused Product
indicates the presence or absence of the at least seven polynucleotide analytes, in any combination of presence or absence,	Chapter 1 Introduction to Droplet Digital PCR Droplet Digital™ PCR is a digital polymerase chain reaction method based on water-oil emulsion droplet technology. Droplet Digital PCR™ (ddPCR™) uses a combination of microfluidics and proprietary surfactant chemistries to divide each sample into water-in-oil droplets. The technology uses reagents and workflows similar to those used for most standard TaqMan probe-based assays, and provides absolute quantification of nucleic acid target sequences by counting nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions. ddPCR is highly effective in the following areas: ■ Absolute quantification — ddPCR provides a concentration of target DNA copies per input sample without the need for running standard curves, making this technique ideal for target DNA measurements, viral load analysis, and microbial quantification. ■ Genomic alterations such as gene copy number variation (CNV) — CNVs result in too few or too many dosage-sensitive genes responsible for phenotypic variability, complex behavioral traits, and disease. ddPCR enables measurement of 1.2x differences in gene copy number. ■ Detection of rare sequences — researchers must amplify single genes in a complex sample, such as a few tumor cells in a wild-type background. ddPCR is sensitive enough to detect rare mutations or sequences. ■ Gene expression and microRNA analysis — ddPCR provides stand-alone absolute quantification of expression levels, especially low-abundance microRNAs, with sensitivity and precision. ■ Next-generation sequencing (NGS) — ddPCR quantifies NGS sample library preparations to increase sequencing accuracy and reduce run repeats. Validate sequencing results such as single nucleotide polymorphisms or copy number variations with absolute quantification. ■ Single cell analysis — the high degree (10-fold to 100-fold) of cell-cell variation in gene expression and genomic content among homogeneous post-mitotic, progenitor, and stem cell populations drives a need for analysis from single cells. ddPCR enables low copy number quantification and gene expression of individual cells. ■ Genome edit detection — ddPCR enables fast, precise, and cost-effective assessment of HDR (Homology directed repair) and NHEJ (non-homologous end joining) generated by CRISPR-Cas9 or other genome editing tools.

Claims	Exemplary Disclosure of Accused Product
	ddPCR has the following benefits for nucleic acid quantification: ■ Unparalleled precision — The massive sample partitioning afforded by ddPCR enables small fold differences in target DNA sequence between samples to be reliably measured. ■ Increased signal-to-noise ratio — High-copy templates and background are diluted, effectively enriching template concentration in target-positive partitions. This allows for the sensitive detection of rare targets and enables a $\pm 10\%$ precision in quantification. ■ Removal of PCR efficiency bias — Error rates are reduced by removing the amplification efficiency reliance of qPCR, enabling accurate quantification of targets to near zero. ■ Simplified quantification — There is no requirement for a standard curve for absolute quantification. 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.

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. PCR Amplification After droplet generation, the plates are moved through the internal thermal cycler, which performs repeated heating and cooling processes. This amplifies one or more copies of a particular DNA segment, thereby multiplying strands of DNA sequencing into the thousands or millions. Droplet Reader Following PCR amplification of the nucleic acid target in the droplets, the plate moves to the Droplet Reader, which analyzes each droplet individually using a four-color detection system, depending on the number of channels specified in the experiment. The autosampler in the Droplet Reader picks up the droplets from each well in the plate. The droplets are spaced out individually for fluorescence reading and fluorescence is then measured in each droplet. Positive droplets, which contain at least one copy of the target DNA molecule, exhibit increased fluorescence compared to negative droplets and are presented in color in the analysis displays. Negative droplets are presented in grayscale. BIO_CC00006554 at 6572-6574.

Claims	Exemplary Disclosure of Accused Product																
	Experiment Types QX ONE Software offers the experiment types described in Table 9, each enabling different assay options and analysis tools. You can assign multiple experiment types across the plate, but you can assign only one experiment type to each well. Table 9. Experiment types <table><tr><th>Type</th><th>Description</th></tr><tr><td>Direct quantification (DQ)</td><td>DQ uses absolute quantification to determine the concentration (copies/ul) of target DNA copies in a sample. DQ measures the number of positive and negative droplets for each target in a well based on their fluorescence amplitude, and uses a Poisson algorithm to calculate the starting concentration of each target DNA molecule. Note: The ABS experiment type created in QuantaSoft Software versions 1.4 through 1.7 is automatically mapped into the software as a DQ experiment type.</td></tr><tr><td>Copy number variation (CNV)</td><td>CNV determines concentration (as with DQ) and calculates the copy number of an unknown (CNV) target relative to a known reference or references within the same well.</td></tr><tr><td>Mutation detection (MUT)</td><td>MUT determines concentration (as with DQ) and calculates the fractional abundance of an unknown mutant present at a low frequency in a wild-type background.</td></tr><tr><td>Rare event detection (RED)</td><td>RED determines the concentration of a known mutant or a rare target species relative to a given reference species within a large pool of background DNA.</td></tr><tr><td>Drop-off (DOF)</td><td>DOF determines absolute quantification of targets for assays designed to detect non-wild type sequences, such as indels and genome edits. DOF is designed to support an assay strategy where one probe counts all alleles and one 'drop-off' probe sits on top of a predicted cut site or mutation site.</td></tr><tr><td>Gene expression (GEX)</td><td>GEX determines concentration (as with DQ) and calculates relative expression levels of an unknown target relative to a known reference or references within the same well.</td></tr><tr><td>Residual DNA quantification (RDQ)</td><td>RDQ provides a precise method for residual host cell DNA quantification. Note: Users can enter a conversion factor to calculate the mass for a well, up to three decimals. The software displays the result in the Molecular Weight column of the Data Table.</td></tr></table>	Type	Description	Direct quantification (DQ)	DQ uses absolute quantification to determine the concentration (copies/ul) of target DNA copies in a sample. DQ measures the number of positive and negative droplets for each target in a well based on their fluorescence amplitude, and uses a Poisson algorithm to calculate the starting concentration of each target DNA molecule. Note: The ABS experiment type created in QuantaSoft Software versions 1.4 through 1.7 is automatically mapped into the software as a DQ experiment type.	Copy number variation (CNV)	CNV determines concentration (as with DQ) and calculates the copy number of an unknown (CNV) target relative to a known reference or references within the same well.	Mutation detection (MUT)	MUT determines concentration (as with DQ) and calculates the fractional abundance of an unknown mutant present at a low frequency in a wild-type background.	Rare event detection (RED)	RED determines the concentration of a known mutant or a rare target species relative to a given reference species within a large pool of background DNA.	Drop-off (DOF)	DOF determines absolute quantification of targets for assays designed to detect non-wild type sequences, such as indels and genome edits. DOF is designed to support an assay strategy where one probe counts all alleles and one 'drop-off' probe sits on top of a predicted cut site or mutation site.	Gene expression (GEX)	GEX determines concentration (as with DQ) and calculates relative expression levels of an unknown target relative to a known reference or references within the same well.	Residual DNA quantification (RDQ)	RDQ provides a precise method for residual host cell DNA quantification. Note: Users can enter a conversion factor to calculate the mass for a well, up to three decimals. The software displays the result in the Molecular Weight column of the Data Table.
Type	Description																
Direct quantification (DQ)	DQ uses absolute quantification to determine the concentration (copies/ul) of target DNA copies in a sample. DQ measures the number of positive and negative droplets for each target in a well based on their fluorescence amplitude, and uses a Poisson algorithm to calculate the starting concentration of each target DNA molecule. Note: The ABS experiment type created in QuantaSoft Software versions 1.4 through 1.7 is automatically mapped into the software as a DQ experiment type.																
Copy number variation (CNV)	CNV determines concentration (as with DQ) and calculates the copy number of an unknown (CNV) target relative to a known reference or references within the same well.																
Mutation detection (MUT)	MUT determines concentration (as with DQ) and calculates the fractional abundance of an unknown mutant present at a low frequency in a wild-type background.																
Rare event detection (RED)	RED determines the concentration of a known mutant or a rare target species relative to a given reference species within a large pool of background DNA.																
Drop-off (DOF)	DOF determines absolute quantification of targets for assays designed to detect non-wild type sequences, such as indels and genome edits. DOF is designed to support an assay strategy where one probe counts all alleles and one 'drop-off' probe sits on top of a predicted cut site or mutation site.																
Gene expression (GEX)	GEX determines concentration (as with DQ) and calculates relative expression levels of an unknown target relative to a known reference or references within the same well.																
Residual DNA quantification (RDQ)	RDQ provides a precise method for residual host cell DNA quantification. Note: Users can enter a conversion factor to calculate the mass for a well, up to three decimals. The software displays the result in the Molecular Weight column of the Data Table.																
	BIO_CC00006554 at 6618.																

Claims	Exemplary Disclosure of Accused Product														
	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. <table><tr><th>ddPCR Supermix</th><th>Description</th></tr><tr><td>Multiplex Supermix</td><td>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.</td></tr><tr><td>Supermix for Probes (No dUTP)</td><td>For use in nucleic acid sample preparations, with sensitivity for the amplification and detection of DNA targets using hydrolysis probe-based assays.</td></tr><tr><td>Supermix for Probes</td><td>For use in sample preparation with uracil N-glycosylase (UNG) decontamination protocols to prevent the reamplification of carryover PCR products between experiments.</td></tr><tr><td>Supermix for Residual DNA Quantification</td><td>For use in residual DNA detection.</td></tr><tr><td>One-Step RT Advanced Kit for Probes</td><td>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.</td></tr><tr><td>EvaGreen® Supermix</td><td>For use in gene expression.</td></tr></table>	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-glycosylase (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.
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-glycosylase (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.														

Claims	Exemplary Disclosure of Accused Product
	Using the One Step RT ddPCR Advanced Kit for Probes The One Step RT ddPCR Advance Kit for Probes supermix is time and temperature-sensitive. Therefore, if you are performing an experiment using this supermix, it is important that the plate does not sit idle in one of the Inbox slots. To perform an experiment using this kit 1. Wait until all plates in the QX ONE Droplet Digital PCR System have processed and then place the One Step RT plate into the first slot. 2. Quickly set up and start the run. For information, see Plate Configuration Window on page 56 and Running Experiments on page 71 Running Experiments on page 71.

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.

Table 10. Assay types, continued

Assay type	Description
Double drop-off	This assay type is available only for the DOF experiment type, and allows two groups of three targets each. The first group appears by default, and looks for one target in FAM <u>and</u> in HEX/VIC (target type reference) one target in FAM (target type unknown), and one target in HEX/VIC (target type unknown). You can add the second group to look for one target in Cy5 <u>and</u> Cy5.5 (target type reference, with two reference copies), one target in Cy5 (target type unknown), and one target in Cy5.5 (target type unknown). By default, all reference copies are set to 2. The channels are static for each group. (Channel 1 FAM is always paired with Channel 2 HEX/VIC, Channel 3 Cy5 is always paired with Channel 4 Cy5.5 .

The experiment type you select determines the available assay methods. [Table 11](#) displays the sample type associated with each assay method.

Table 11. Assay types by experiment type

Assay type	Experiment type						
	DQ	CNV	MUT	DOF	GEX	RDQ	RED
Single target per channel	✓	✓	✓		✓	✓	✓
Amplitude multiplex	✓	✓	✓		✓	✓	✓
Probe mix triplex	✓	✓	✓		✓	✓	✓
Advanced classification	✓						✓
Basic drop-off				✓			
Double drop-off				✓			

QX ONE Software automatically populates the fluorophore in each channel for each target but you can change the default selections.

Use [Table 12](#) to select from the fluorophore options for each assay method listed.

Claims

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Table 12. Fluorophore options

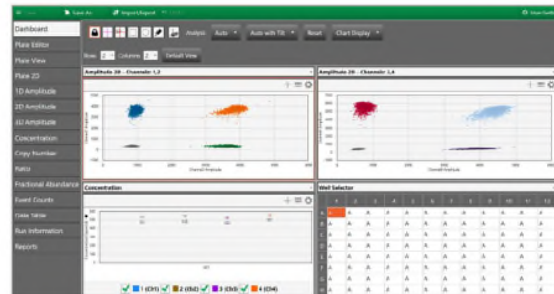
Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4
Single target per channel, for 1 to 4 targets	FAM	HEX	Cy5	Cy5.5
	None	VIC	None	None
		None		
Amplitude multiplex, for 1 to 8 targets	FAM Hi	HEX Hi	Cy5 Hi	Cy5.5 Hi
	FAM Lo	HEX Lo	Cy 5 Lo	Cy 5.5 Lo
	None	VIC Hi	None	None
		VIC Lo		
		None		
Probe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

BIO_CC00006554 at 6620-6624.

All determinations are shown as a unique color pattern on the analysis dashboard.

Analysis Dashboard

When you open a data file, the Analysis module opens to a Dashboard window containing default analysis and data panes in a summary view. You can customize the view to change panes, or increase or decrease the number of panes. For information on the available options in the Dashboard view, see [Dashboard Options on page 84](#). For more information on the Analysis Dashboard, refer to the QX ONE Software User Guide.



Note: If you open a .qlo file, the Plate Editor opens as the default view and you must select the Dashboard tab to open it.

From the tabs on the left, you can access plate views, amplitude charts, data visualization distribution charts, droplet counts, and a complete data table. You can also view data specific to the run in the Run Information window, and run reports on any of the analysis charts from the Reports window.

Dashboard Options

From the Dashboard window, depending on the individual dashboard window you have selected, you can use a range of options as described below:

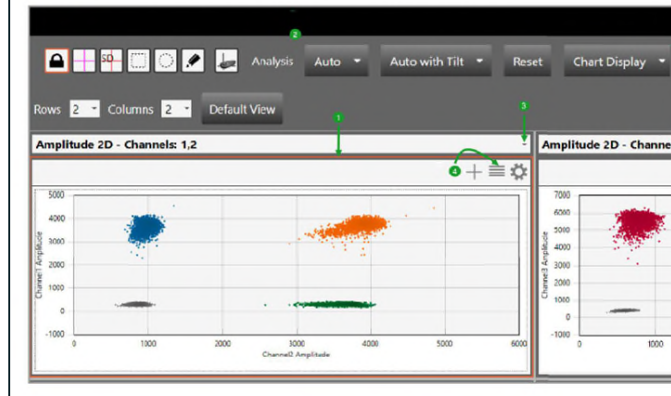
- When you select a window, the options that appear in the menu bar correspond to the options you see if you open the same window from the side menu. For example, if you select the Concentration chart in the Dashboard, the Concentration menu options appear.



Claims

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Table 15 describes the option sets identified in the following graphic.



BIO_CCODE00006652- BIO_CCODE00006654.

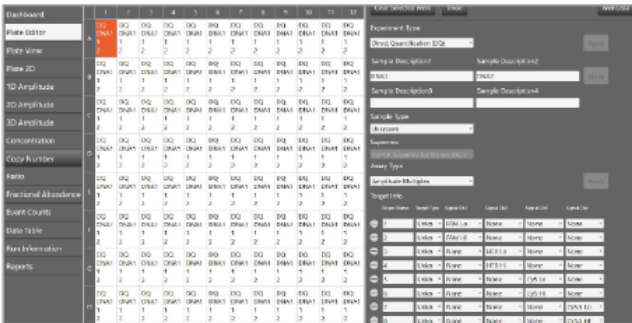
Claims

Exemplary Disclosure of Accused Product

Setting Up Experiment Parameters

To set experiment parameters

1. Open the analysis data file and select the Plate Editor tab.



2. Select one or more wells to be assigned well analysis attributes.
3. Change the experiment type to Direct Quantification (DQ), and then tap Apply.
4. (Optional) Change the sample description identifiers.
5. (Optional) Change the sample type. The default type is Unknown.
- Tip:** For automatic thresholding of positive control wells, you must select Pos Ctrl.
6. Under Assay Type, select Amplitude Multiplex and tap Apply. Eight target rows appear.
7. Change the target name and type (unknown or reference) for each target in the well.
- The software defaults to FAM Lo, FAM Hi, HEX Lo, HEX Hi, Cy5 Lo, Cy5 Hi, Cy5.5 Lo, and Cy5.5 Hi, respectively, for each target.

BIO CCODE00007041 at 7212.

The QX ONE defaults to FAM Lo, FAM Hi, HEX Lo, HEX Hi, Cy5 Lo, Cy5 Hi, Cy5.5 Lo, Cy5.5 Hi, ROX Lo, ROX Hi, ATTO 590 Lo, and ATTO 590 Hi, respectively, for each target. Thus, on information and belief, each target is encoded with a specific fluorophore corresponding to a cumulative signal measurement to non-degenerately indicate the presence or absence of each target.

Claims	Exemplary Disclosure of Accused Product
	When the QX ONE is used, each cumulative signal measurement gives a unique pattern that, on information and belief, non-degenerately indicates the presence or absence of the at least seven polynucleotide analytes, in any combination of presence or absence. On information and belief, this may be based on the specific encoding scheme chosen.
[1d.1] wherein said method non-degenerately detects the presence or absence of the at least seven polynucleotide analytes in said single sample solution volume, in any combination of presence or absence	Plaintiffs incorporate their discussion from steps 1a, 1b, and 1c in their entirety. As discussed in those steps, the presence or absence of the at least seven polynucleotide analytes the sample is detected non-degenerately. Further: The QX One System is used to perform the step of non-degenerately detects the presence or absence of the at least seven polynucleotide analytes in said single sample solution volume, in any combination of presence or absence, either literally or under the doctrine of equivalents. The signals non-degenerately indicates the presence or absence of the targeted analytes, based on the experiment types.

Claims	Exemplary Disclosure of Accused Product
	Chapter 1 Introduction to Droplet Digital PCR Droplet Digital™ PCR is a digital polymerase chain reaction method based on water-oil emulsion droplet technology. Droplet Digital PCR™ (ddPCR™) uses a combination of microfluidics and proprietary surfactant chemistries to divide each sample into water-in-oil droplets. The technology uses reagents and workflows similar to those used for most standard TaqMan probe-based assays, and provides absolute quantification of nucleic acid target sequences by counting nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions. ddPCR is highly effective in the following areas: ■ Absolute quantification — ddPCR provides a concentration of target DNA copies per input sample without the need for running standard curves, making this technique ideal for target DNA measurements, viral load analysis, and microbial quantification. ■ Genomic alterations such as gene copy number variation (CNV) — CNVs result in too few or too many dosage-sensitive genes responsible for phenotypic variability, complex behavioral traits, and disease. ddPCR enables measurement of 1.2x differences in gene copy number. ■ Detection of rare sequences — researchers must amplify single genes in a complex sample, such as a few tumor cells in a wild-type background. ddPCR is sensitive enough to detect rare mutations or sequences. ■ Gene expression and microRNA analysis — ddPCR provides stand-alone absolute quantification of expression levels, especially low-abundance microRNAs, with sensitivity and precision. ■ Next-generation sequencing (NGS) — ddPCR quantifies NGS sample library preparations to increase sequencing accuracy and reduce run repeats. Validate sequencing results such as single nucleotide polymorphisms or copy number variations with absolute quantification. ■ Single cell analysis — the high degree (10-fold to 100-fold) of cell-cell variation in gene expression and genomic content among homogeneous post-mitotic, progenitor, and stem cell populations drives a need for analysis from single cells. ddPCR enables low copy number quantification and gene expression of individual cells. ■ Genome edit detection — ddPCR enables fast, precise, and cost-effective assessment of HDR (Homology directed repair) and NHEJ (non-homologous end joining) generated by CRISPR-Cas9 or other genome editing tools.

Claims	Exemplary Disclosure of Accused Product
	ddPCR has the following benefits for nucleic acid quantification: ■ Unparalleled precision — The massive sample partitioning afforded by ddPCR enables small fold differences in target DNA sequence between samples to be reliably measured. ■ Increased signal-to-noise ratio — High-copy templates and background are diluted, effectively enriching template concentration in target-positive partitions. This allows for the sensitive detection of rare targets and enables a $\pm 10\%$ precision in quantification. ■ Removal of PCR efficiency bias — Error rates are reduced by removing the amplification efficiency reliance of qPCR, enabling accurate quantification of targets to near zero. ■ Simplified quantification — There is no requirement for a standard curve for absolute quantification. 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.

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. PCR Amplification After droplet generation, the plates are moved through the internal thermal cycler, which performs repeated heating and cooling processes. This amplifies one or more copies of a particular DNA segment, thereby multiplying strands of DNA sequencing into the thousands or millions. Droplet Reader Following PCR amplification of the nucleic acid target in the droplets, the plate moves to the Droplet Reader, which analyzes each droplet individually using a four-color detection system, depending on the number of channels specified in the experiment. The autosampler in the Droplet Reader picks up the droplets from each well in the plate. The droplets are spaced out individually for fluorescence reading and fluorescence is then measured in each droplet. Positive droplets, which contain at least one copy of the target DNA molecule, exhibit increased fluorescence compared to negative droplets and are presented in color in the analysis displays. Negative droplets are presented in grayscale. BIO_CC00006554 at 6572-6574.

Claims	Exemplary Disclosure of Accused Product																
	Experiment Types QX ONE Software offers the experiment types described in Table 9, each enabling different assay options and analysis tools. You can assign multiple experiment types across the plate, but you can assign only one experiment type to each well. Table 9. Experiment types <table><tr><th>Type</th><th>Description</th></tr><tr><td>Direct quantification (DQ)</td><td>DQ uses absolute quantification to determine the concentration (copies/ul) of target DNA copies in a sample. DQ measures the number of positive and negative droplets for each target in a well based on their fluorescence amplitude, and uses a Poisson algorithm to calculate the starting concentration of each target DNA molecule. Note: The ABS experiment type created in QuantaSoft Software versions 1.4 through 1.7 is automatically mapped into the software as a DQ experiment type.</td></tr><tr><td>Copy number variation (CNV)</td><td>CNV determines concentration (as with DQ) and calculates the copy number of an unknown (CNV) target relative to a known reference or references within the same well.</td></tr><tr><td>Mutation detection (MUT)</td><td>MUT determines concentration (as with DQ) and calculates the fractional abundance of an unknown mutant present at a low frequency in a wild-type background.</td></tr><tr><td>Rare event detection (RED)</td><td>RED determines the concentration of a known mutant or a rare target species relative to a given reference species within a large pool of background DNA.</td></tr><tr><td>Drop-off (DOF)</td><td>DOF determines absolute quantification of targets for assays designed to detect non-wild type sequences, such as indels and genome edits. DOF is designed to support an assay strategy where one probe counts all alleles and one 'drop-off' probe sits on top of a predicted cut site or mutation site.</td></tr><tr><td>Gene expression (GEX)</td><td>GEX determines concentration (as with DQ) and calculates relative expression levels of an unknown target relative to a known reference or references within the same well.</td></tr><tr><td>Residual DNA quantification (RDQ)</td><td>RDQ provides a precise method for residual host cell DNA quantification. Note: Users can enter a conversion factor to calculate the mass for a well, up to three decimals. The software displays the result in the Molecular Weight column of the Data Table.</td></tr></table>	Type	Description	Direct quantification (DQ)	DQ uses absolute quantification to determine the concentration (copies/ul) of target DNA copies in a sample. DQ measures the number of positive and negative droplets for each target in a well based on their fluorescence amplitude, and uses a Poisson algorithm to calculate the starting concentration of each target DNA molecule. Note: The ABS experiment type created in QuantaSoft Software versions 1.4 through 1.7 is automatically mapped into the software as a DQ experiment type.	Copy number variation (CNV)	CNV determines concentration (as with DQ) and calculates the copy number of an unknown (CNV) target relative to a known reference or references within the same well.	Mutation detection (MUT)	MUT determines concentration (as with DQ) and calculates the fractional abundance of an unknown mutant present at a low frequency in a wild-type background.	Rare event detection (RED)	RED determines the concentration of a known mutant or a rare target species relative to a given reference species within a large pool of background DNA.	Drop-off (DOF)	DOF determines absolute quantification of targets for assays designed to detect non-wild type sequences, such as indels and genome edits. DOF is designed to support an assay strategy where one probe counts all alleles and one 'drop-off' probe sits on top of a predicted cut site or mutation site.	Gene expression (GEX)	GEX determines concentration (as with DQ) and calculates relative expression levels of an unknown target relative to a known reference or references within the same well.	Residual DNA quantification (RDQ)	RDQ provides a precise method for residual host cell DNA quantification. Note: Users can enter a conversion factor to calculate the mass for a well, up to three decimals. The software displays the result in the Molecular Weight column of the Data Table.
Type	Description																
Direct quantification (DQ)	DQ uses absolute quantification to determine the concentration (copies/ul) of target DNA copies in a sample. DQ measures the number of positive and negative droplets for each target in a well based on their fluorescence amplitude, and uses a Poisson algorithm to calculate the starting concentration of each target DNA molecule. Note: The ABS experiment type created in QuantaSoft Software versions 1.4 through 1.7 is automatically mapped into the software as a DQ experiment type.																
Copy number variation (CNV)	CNV determines concentration (as with DQ) and calculates the copy number of an unknown (CNV) target relative to a known reference or references within the same well.																
Mutation detection (MUT)	MUT determines concentration (as with DQ) and calculates the fractional abundance of an unknown mutant present at a low frequency in a wild-type background.																
Rare event detection (RED)	RED determines the concentration of a known mutant or a rare target species relative to a given reference species within a large pool of background DNA.																
Drop-off (DOF)	DOF determines absolute quantification of targets for assays designed to detect non-wild type sequences, such as indels and genome edits. DOF is designed to support an assay strategy where one probe counts all alleles and one 'drop-off' probe sits on top of a predicted cut site or mutation site.																
Gene expression (GEX)	GEX determines concentration (as with DQ) and calculates relative expression levels of an unknown target relative to a known reference or references within the same well.																
Residual DNA quantification (RDQ)	RDQ provides a precise method for residual host cell DNA quantification. Note: Users can enter a conversion factor to calculate the mass for a well, up to three decimals. The software displays the result in the Molecular Weight column of the Data Table.																
	BIO_CC00006554 at 6618.																

Claims	Exemplary Disclosure of Accused Product														
	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. <table> <tr> <th>ddPCR Supermix</th><th>Description</th></tr> <tr> <td>Multiplex Supermix</td><td>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.</td></tr> <tr> <td>Supermix for Probes (No dUTP)</td><td>For use in nucleic acid sample preparations, with sensitivity for the amplification and detection of DNA targets using hydrolysis probe-based assays.</td></tr> <tr> <td>Supermix for Probes</td><td>For use in sample preparation with uracil N-glycosylase (UNG) decontamination protocols to prevent the reamplification of carryover PCR products between experiments.</td></tr> <tr> <td>Supermix for Residual DNA Quantification</td><td>For use in residual DNA detection.</td></tr> <tr> <td>One-Step RT Advanced Kit for Probes</td><td>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.</td></tr> <tr> <td>EvaGreen® Supermix</td><td>For use in gene expression.</td></tr> </table>	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-glycosylase (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.
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-glycosylase (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.														

Claims	Exemplary Disclosure of Accused Product
	Using the One Step RT ddPCR Advanced Kit for Probes The One Step RT ddPCR Advance Kit for Probes supermix is time and temperature-sensitive. Therefore, if you are performing an experiment using this supermix, it is important that the plate does not sit idle in one of the Inbox slots. To perform an experiment using this kit 1. Wait until all plates in the QX ONE Droplet Digital PCR System have processed and then place the One Step RT plate into the first slot. 2. Quickly set up and start the run. For information, see Plate Configuration Window on page 56 and Running Experiments on page 71 Running Experiments on page 71.

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 <table> <tr> <th>Assay type</th><th>Description</th></tr> <tr> <td>Single target per channel</td><td>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.</td></tr> <tr> <td>Amplitude multiplex</td><td>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.</td></tr> <tr> <td>Probe mix triplex</td><td>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.</td></tr> <tr> <td>Advanced classification</td><td>Method to increase multiplexing rate in any combination of targets detected per channel. This method requires manual classification of droplets.</td></tr> <tr> <td>Basic drop-off</td><td>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.</td></tr> </table>	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.
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.												

Table 10. Assay types, continued

Assay type	Description
Double drop-off	This assay type is available only for the DOF experiment type, and allows two groups of three targets each. The first group appears by default, and looks for one target in FAM <u>and</u> in HEX/VIC (target type reference) one target in FAM (target type unknown), and one target in HEX/VIC (target type unknown). You can add the second group to look for one target in Cy5 <u>and</u> Cy5.5 (target type reference, with two reference copies), one target in Cy5 (target type unknown), and one target in Cy5.5 (target type unknown). By default, all reference copies are set to 2. The channels are static for each group. (Channel 1 FAM is always paired with Channel 2 HEX/VIC, Channel 3 Cy5 is always paired with Channel 4 Cy5.5 .

The experiment type you select determines the available assay methods. [Table 11](#) displays the sample type associated with each assay method.

Table 11. Assay types by experiment type

Assay type	Experiment type						
	DQ	CNV	MUT	DOF	GEX	RDQ	RED
Single target per channel	✓	✓	✓		✓	✓	✓
Amplitude multiplex	✓	✓	✓		✓	✓	✓
Probe mix triplex	✓	✓	✓		✓	✓	✓
Advanced classification	✓						✓
Basic drop-off				✓			
Double drop-off				✓			

QX ONE Software automatically populates the fluorophore in each channel for each target but you can change the default selections.

Use [Table 12](#) to select from the fluorophore options for each assay method listed.

Claims

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Table 12. Fluorophore options

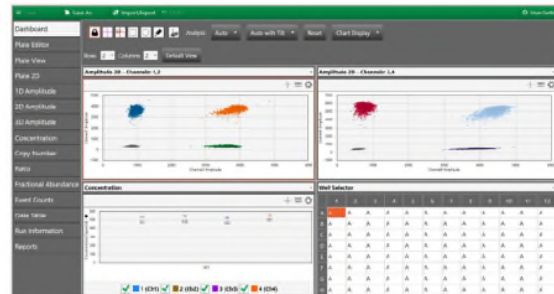
Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4
Single target per channel, for 1 to 4 targets	FAM	HEX	Cy5	Cy5.5
	None	VIC	None	None
		None		
Amplitude multiplex, for 1 to 8 targets	FAM Hi	HEX Hi	Cy5 Hi	Cy5.5 Hi
	FAM Lo	HEX Lo	Cy 5 Lo	Cy 5.5 Lo
	None	VIC Hi	None	None
		VIC Lo		
		None		
Probe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

BIO_CC00006554 at 6620-6624.

All determinations are shown as a unique color pattern on the analysis dashboard.

Analysis Dashboard

When you open a data file, the Analysis module opens to a Dashboard window containing default analysis and data panes in a summary view. You can customize the view to change panes, or increase or decrease the number of panes. For information on the available options in the Dashboard view, see [Dashboard Options on page 84](#). For more information on the Analysis Dashboard, refer to the QX ONE Software User Guide.



Note: If you open a .qlo file, the Plate Editor opens as the default view and you must select the Dashboard tab to open it.

From the tabs on the left, you can access plate views, amplitude charts, data visualization distribution charts, droplet counts, and a complete data table. You can also view data specific to the run in the Run Information window, and run reports on any of the analysis charts from the Reports window.

Dashboard Options

From the Dashboard window, depending on the individual dashboard window you have selected, you can use a range of options as described below:

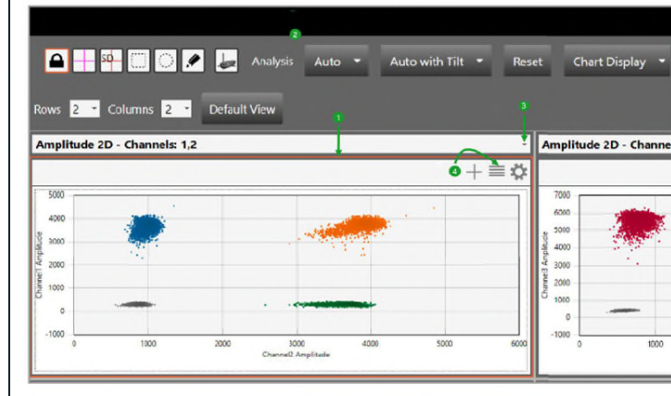
- When you select a window, the options that appear in the menu bar correspond to the options you see if you open the same window from the side menu. For example, if you select the Concentration chart in the Dashboard, the Concentration menu options appear.



Claims

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Table 15 describes the option sets identified in the following graphic.



BIO_CCODE00006652- BIO_CCODE00006654.

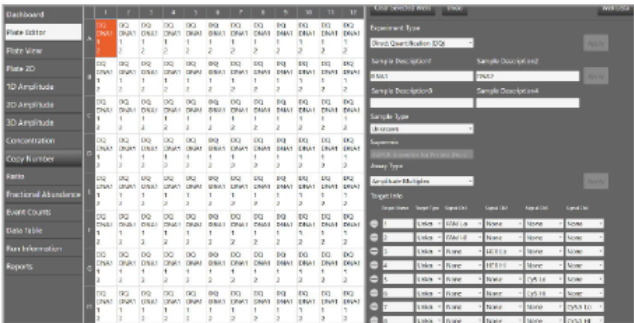
Claims

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Setting Up Experiment Parameters

To set experiment parameters

- 1. Open the analysis data file and select the Plate Editor tab.



- 2. Select one or more wells to be assigned well analysis attributes.
 - 3. Change the experiment type to Direct Quantification (DQ), and then tap Apply.
 - 4. (Optional) Change the sample description identifiers.
 - 5. (Optional) Change the sample type. The default type is Unknown.
 - Tip:** For automatic thresholding of positive control wells, you must select Pos Ctrl.
 - 6. Under Assay Type, select Amplitude Multiplex and tap Apply. Eight target rows appear.
 - 7. Change the target name and type (unknown or reference) for each target in the well.
- The software defaults to FAM Lo, FAM Hi, HEX Lo, HEX Hi, Cy5 Lo, Cy5 Hi, Cy5.5 Lo, and Cy5.5 Hi, respectively, for each target.

BIO CCODE00007041 at 7212.

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 <u>and</u> HEX/VIC and one target in FAM <u>or</u> 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 <u>and</u> Cy5.5, one target in Cy5 <u>or</u> 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.

Table 10. Assay types, continued

Assay type	Description
Double drop-off	This assay type is available only for the DOF experiment type, and allows two groups of three targets each. The first group appears by default, and looks for one target in FAM and in HEX/VIC (target type reference) one target in FAM (target type unknown), and one target in HEX/VIC (target type unknown). You can add the second group to look for one target in Cy5 and Cy5.5 (target type reference, with two reference copies), one target in Cy5 (target type unknown), and one target in Cy5.5 (target type unknown). By default, all reference copies are set to 2. The channels are static for each group. (Channel 1 FAM is always paired with Channel 2 HEX/VIC, Channel 3 Cy5 is always paired with Channel 4 Cy5.5..

The experiment type you select determines the available assay methods. [Table 11](#) displays the sample type associated with each assay method.

Table 11. Assay types by experiment type

Assay type	Experiment type						
	DQ	CNV	MUT	DOF	GEX	RDQ	RED
Single target per channel	✓	✓	✓		✓	✓	✓
Amplitude multiplex	✓	✓	✓		✓	✓	✓
Probe mix triplex	✓	✓	✓		✓	✓	✓
Advanced classification	✓						✓
Basic drop-off				✓			
Double drop-off				✓			

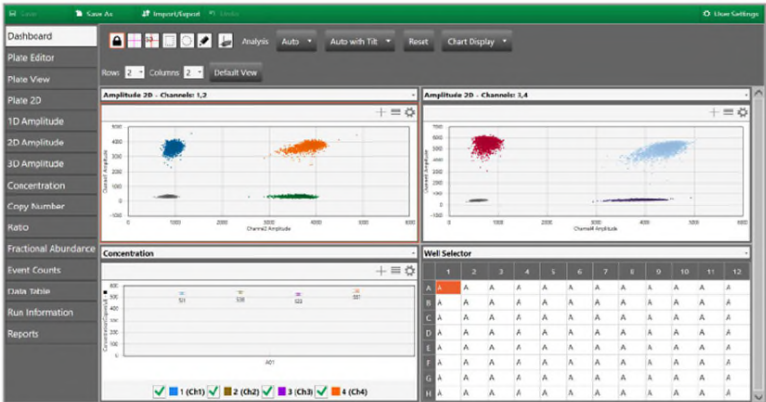
QX ONE Software automatically populates the fluorophore in each channel for each target but you can change the default selections.

Use [Table 12](#) to select from the fluorophore options for each assay method listed.

Claims	Exemplary Disclosure of Accused Product																																																																									
	Table 12. Fluorophore options <table><tr><th>Assay method</th><th>Signal Ch1</th><th>Signal Ch2</th><th>Signal Ch3</th><th>Signal Ch4</th></tr><tr><td rowspan="3">Single target per channel, for 1 to 4 targets</td><td>FAM</td><td>HEX</td><td>Cy5</td><td>Cy5.5</td></tr><tr><td>None</td><td>VIC</td><td>None</td><td>None</td></tr><tr><td></td><td>None</td><td></td><td></td></tr><tr><td rowspan="5">Amplitude multiplex, for 1 to 8 targets</td><td>FAM Hi</td><td>HEX Hi</td><td>Cy5 Hi</td><td>Cy5.5 Hi</td></tr><tr><td>FAM Lo</td><td>HEX Lo</td><td>Cy 5 Lo</td><td>Cy 5.5 Lo</td></tr><tr><td>None</td><td>VIC Hi</td><td>None</td><td>None</td></tr><tr><td></td><td>VIC Lo</td><td></td><td></td></tr><tr><td></td><td>None</td><td></td><td></td></tr><tr><td rowspan="3">Probe mix triplex, for 6 targets</td><td>FAM</td><td>HEX</td><td>Cy5</td><td>Cy5.5</td></tr><tr><td>None</td><td>VIC</td><td>None</td><td>None</td></tr><tr><td></td><td>None</td><td></td><td></td></tr><tr><td rowspan="5">Advanced classification</td><td>FAM</td><td>HEX</td><td>Cy5</td><td>Cy5.5</td></tr><tr><td>+1-10</td><td>1 to 10</td><td>1 to 10</td><td>1 to 10</td></tr><tr><td>None</td><td>VIC</td><td>None</td><td>None</td></tr><tr><td></td><td>1 to 10</td><td></td><td></td></tr><tr><td></td><td>None</td><td></td><td></td></tr></table> BIO_CC00006622-BIO_CC00006624 All determinations are shown as a unique color pattern on the analysis dashboard.	Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4	Single target per channel, for 1 to 4 targets	FAM	HEX	Cy5	Cy5.5	None	VIC	None	None		None			Amplitude multiplex, for 1 to 8 targets	FAM Hi	HEX Hi	Cy5 Hi	Cy5.5 Hi	FAM Lo	HEX Lo	Cy 5 Lo	Cy 5.5 Lo	None	VIC Hi	None	None		VIC Lo				None			Probe mix triplex, for 6 targets	FAM	HEX	Cy5	Cy5.5	None	VIC	None	None		None			Advanced classification	FAM	HEX	Cy5	Cy5.5	+1-10	1 to 10	1 to 10	1 to 10	None	VIC	None	None		1 to 10				None		
Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4																																																																						
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	None	VIC	None	None																																																																						
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		None																																																																								

Analysis Dashboard

When you open a data file, the Analysis module opens to a Dashboard window containing default analysis and data panes in a summary view. You can customize the view to change panes, or increase or decrease the number of panes. For information on the available options in the Dashboard view, see [Dashboard Options on page 84](#). For more information on the Analysis Dashboard, refer to the QX ONE Software User Guide.



Note: If you open a .qlp file, the Plate Editor opens as the default view and you must select the Dashboard tab to open it.

From the tabs on the left, you can access plate views, amplitude charts, data visualization distribution charts, droplet counts, and a complete data table. You can also view data specific to the run in the Run Information window, and run reports on any of the analysis charts from the Reports window.

Dashboard Options

From the Dashboard window, depending on the individual dashboard window you have selected, you can use a range of options as described below:

- When you select a window, the options that appear in the menu bar correspond to the options you see if you open the same window from the side menu. For example, if you select the Concentration chart in the Dashboard, the Concentration menu options appear.

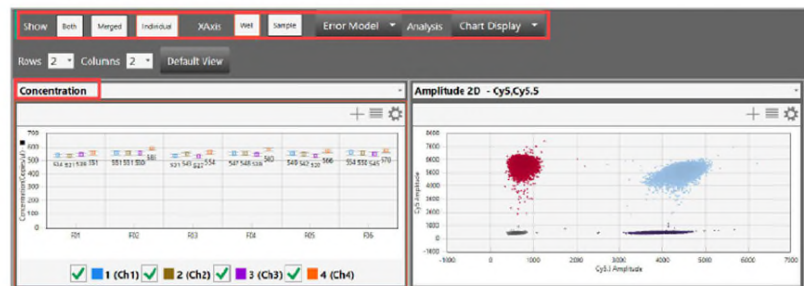
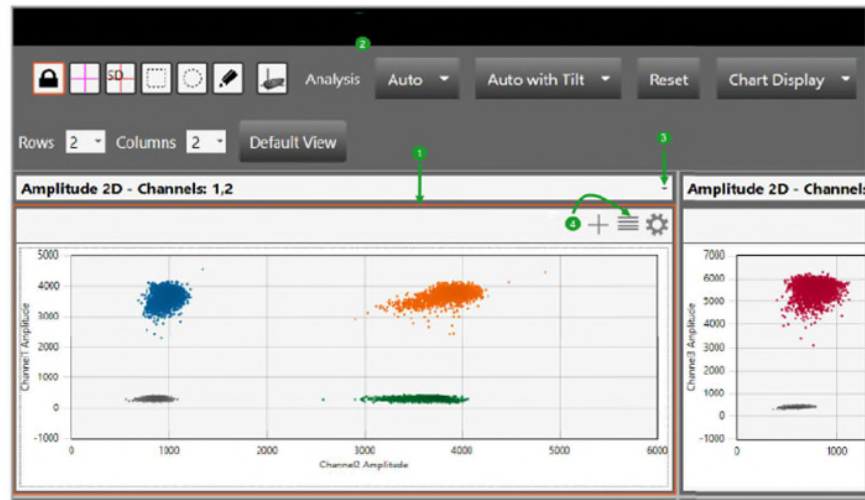


Table 15 describes the option sets identified in the following graphic.



BIO_CC00006652- BIO_CC00006654.

The QX ONE System defaults to FAM Lo, FAM Hi, HEX Lo, HEX Hi, Cy5 Lo, Cy5 Hi, Cy5.5 Lo, Cy5.5 Hi, ROX Lo, ROX Hi, ATTO 590 Lo, and ATTO 590 Hi, respectively, for each target. Thus, on information and belief, each target is encoded with a specific fluorophore corresponding to a cumulative signal measurement to non-degenerately indicate the presence or absence of each target.

Claims	Exemplary Disclosure of Accused Product
[1d.2] without requiring any step of immobilization of said polynucleotide analytes, physical separation of said polynucleotide analytes, or mass spectrometry.	Plaintiffs incorporates their discussion from steps 1a, 1b, and 1c in their entirety. As discussed in those steps, the presence or absence of the at least seven polynucleotide analytes the sample is detected non-degenerately. Also, as shown above, no immobilizing or physical separation of any polynucleotide analytes occurs. Likewise, no mass spectrometry is performed. The QX One System is used to perform the step that without requiring any step of immobilization of said polynucleotide analytes, physical separation of said polynucleotide analytes, or mass spectrometry, either literally or under the doctrine of equivalents. The QX One System follows the ddPCR Workflow which fractionates a sample into 20,000 nanoliter-sized droplets with target and background DNA distributed randomly into droplets during the partitioning process. 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.

Claims	Exemplary Disclosure of Accused Product						
	BIO_CC00006573						
2. The method of claim 1, wherein said hybridization probes comprise an identical fluorophore, and wherein said plurality of hybridization probes are specific for different polynucleotide analytes.	Plaintiffs incorporate their analysis from Claim 1 here. The QX One System is used to perform the step of wherein said hybridization probes comprise an identical fluorophore, and wherein said plurality of hybridization probes are specific for different polynucleotide analytes, either literally or under the doctrine of equivalents. Under Amplitude Multiplex, the QX One System can detect up to two targets per channel. For example, using an identical fluorophore (FAM), two different analytes can be detected. One corresponds to FAM Lo and the other corresponds to FAM Hi. The intensity differences for an identical fluorophore allows for said plurality of hybridization probes comprising an identical fluorophore to be contained in a probe that is specific for different polynucleotide analytes. Assay Types and Fluorophores QX ONE Software offers the assay methods explained in Table 10. Table 10. Assay types <table> <tr> <th>Assay type</th><th>Description</th></tr> <tr> <td>Single target per channel</td><td>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.</td></tr> <tr> <td>Amplitude multiplex</td><td>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.</td></tr> </table> BIO_CC00006622	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.
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Assay method	Signal Ch1	Signal Ch2	Signal Ch3	Signal Ch4																																																																						
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	None	VIC	None	None																																																																						
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Amplitude multiplex, for 1 to 8 targets	FAM Hi	HEX Hi	Cy5 Hi	Cy5.5 Hi																																																																						
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Probe mix triplex, for 6 targets	FAM	HEX	Cy5	Cy5.5																																																																						
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	None	VIC	None	None																																																																						
		1 to 10																																																																								
		None																																																																								
3. The method of claim 1, wherein said detecting is performed with a plurality of hybridization probes comprising different fluorophores, wherein said plurality of hybridization probes	Plaintiffs incorporate by reference their discussion from Claim 1, including in particular claimed steps 1a. As discussed there, the QX600 is designed to with a plurality of hybridization probes comprising different fluorophores, wherein said plurality of hybridization probes are specific for different analytes, both literally and under the doctrine of equivalents. The QX One System is used to perform the step of wherein said detecting is performed with a plurality of hybridization probes comprising different fluorophores, wherein said plurality of hybridization probes are specific for different analytes, either literally or under the doctrine of equivalents.																																																																									

Claims	Exemplary Disclosure of Accused Product						
are specific for different analytes.	Under Amplitude Multiplex, the QX One System can detect up to two targets per channel. For example, the four fluorophores (FAM, HEX, Cy5 and Cy5.5) reside on four different channels. Each fluorophores can be used to detect one to two different analytes. Each probe corresponds to one of the polynucleotide analytes. Each of the probes also corresponds to a specific fluorophore or a combination of fluorophores, with each corresponding to a color. Thus, the detection is performed with hybridization probes comprising different fluorophores, wherein the hybridization probes are specific for different analytes. Assay Types and Fluorophores QX ONE Software offers the assay methods explained in Table 10. Table 10. Assay types <table> <tr> <th>Assay type</th><th>Description</th></tr> <tr> <td>Single target per channel</td><td>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.</td></tr> <tr> <td>Amplitude multiplex</td><td>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.</td></tr> </table> BIO_CC00006622	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.
Assay type	Description						
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	None	VIC	None	None																																																																			
		1 to 10																																																																					
		None																																																																					
4. The method of claim 1, wherein said cumulative intensity signal(s) are generated during a polymerase chain reaction.	The QX One System is used to perform the step wherein said cumulative intensity signal(s) are generated during a polymerase chain reaction, either literally or under the doctrine of equivalents. The QX One System is Droplet Digital PCR which is a digital polymerase chain reaction method.																																																																						

Claims	Exemplary Disclosure of Accused Product
	Chapter 1 Introduction to Droplet Digital PCR Droplet Digital TM PCR is a digital polymerase chain reaction method based on water-oil emulsion droplet technology. Droplet Digital PCRTM (ddPCRTM) uses a combination of microfluidics and proprietary surfactant chemistries to divide each sample into water-in-oil droplets. The technology uses reagents and workflows similar to those used for most standard TaqMan probe-based assays, and provides absolute quantification of nucleic acid target sequences by counting nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions. ddPCR is highly effective in the following areas: BIO_CC00006572
5. The method of claim 1, further comprising transmitting information concerning the presence or absence of said polynucleotide analytes through a computer network.	The QX One System is used to perform the step further comprising transmitting information concerning the presence or absence of said polynucleotide analytes through a computer network, either literally or under the doctrine of equivalents.

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. BIO_CC00006554 at 6572. As shown above the QX ONE permits remote access (i.e. transmitting information) concerning the presence or absence of said polynucleotide analytes through a computer network. One such format is CSV. The QX One System allows sharing the CSV files.

Claims	Exemplary Disclosure of Accused Product
	Creating and Storing Plate Templates from CSV Files You can create a plate template in a spreadsheet application, save it in .csv format (CSV), and then store it for use in your droplet reading runs. Note the following: ■ When you create a CSV template, the headings for the columns containing the experiment information must match the fields and order in the Plate Editor. If they do not match, an error message appears when you select the template. ■ After you specify the information in row 1, do not change the field entries until you install a new software version or edition. ■ You can store the CSV file in any location, but the Browse option defaults to your personal templates storage folder defined in your user preferences (see Managing Your Preferences on page 37), or the shared or preferred location specified by your system administrator (see Shared Settings on page 93). ■ CSV templates do not appear in the Templates window. You can select a CSV template file from the Plate Setup dropdown list, or, after you tap Browse, you can navigate to the storage folder and select the file. BIO_CC00006554 at 6642; <i>see also id.</i> at 6576, 6586, 6669, 6671, 6674, 6675, 6676, 6678; <i>see also</i> BIO_CC00007041 at 7054, 7058, 7063, 7064, 7065, 7066, 7109, 7110, 7111, 7181, 7200, 7203, 7205, 7206, 7207, 7209. As another example data analysis and other operations may be performed on a standalone (i.e. remote/networked) computer, but not from the QX One touch screen. <i>See id.</i>
6. The method of claim 1, further comprising providing information concerning the presence or absence of said polynucleotide analytes	On information and belief, after using the QX600, Bio-Rad and/or an end-user provides the information concerning the presence or absence of said polynucleotide analytes to a health care professional, wherein the health care professional makes a clinical decision based on said information, both literally and under the doctrine of equivalents.

Claims	Exemplary Disclosure of Accused Product
to a health care professional, wherein the health care professional makes a clinical decision based on said information.	The QX One System is used to perform the step of comprising providing information concerning the presence or absence of said polynucleotide analytes to a health care professional, wherein the health care professional makes a clinical decision based on said information, either literally or under the doctrine of equivalents. Data that may be shared with a health care profession, may, for example, include raw data such as in a CSV file format: Creating and Storing Plate Templates from CSV Files You can create a plate template in a spreadsheet application, save it in .csv format (CSV), and then store it for use in your droplet reading runs. Note the following: ■ When you create a CSV template, the headings for the columns containing the experiment information must match the fields and order in the Plate Editor. If they do not match, an error message appears when you select the template. ■ After you specify the information in row 1, do not change the field entries until you install a new software version or edition. ■ You can store the CSV file in any location, but the Browse option defaults to your personal templates storage folder defined in your user preferences (see Managing Your Preferences on page 37), or the shared or preferred location specified by your system administrator (see Shared Settings on page 93). ■ CSV templates do not appear in the Templates window. You can select a CSV template file from the Plate Setup dropdown list, or, after you tap Browse, you can navigate to the storage folder and select the file. BIO_CC00006642

Claims	Exemplary Disclosure of Accused Product
7. The method of claim 1, wherein at least one step of said method is performed using a computer.	Plaintiffs incorporate by reference Claims 1 and 5. The QX ONE System itself is a computer. Additionally, the QX ONE system may transmit raw data for analysis (such as generating a cumulative signal measurement) to a remote or networked computer as described above. The QX One System is paired with QX One Software. The screenshot displays two sections of the QX ONE Software documentation. The 'Introduction' section states: 'The QX ONE Droplet Digital PCR System and QX ONE Software provide users with the capability to set up and run up to five plates concurrently through three ddPCR phases (droplet generation, thermal cycling, and droplet reading), and then analyze the results.' The 'Supported Operating System' section states: 'QX ONE Software is supported on the 64-bit Windows 10 operating system.' BIO_CC00006964 The QX One System features a touch screen computer, which is integrated onto the instrument and has QX One Software already installed.

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.

Claims	Exemplary Disclosure of Accused Product
	BIO_CCODE00006576
8. The method of claim 7, wherein said computer is located on a remote server.	Plaintiffs incorporate by reference their analysis in Claims 5 and 7. When a remote computer is connected to the QX ONE as described in those claims, the remote computer to which the QX ONE connects acts as a remote server. The QX One System performs the step wherein said computer is located on a remote server, either literally or under the doctrine of equivalents. The QX One System is paired with QX One Software. Introduction The QX ONE Droplet Digital PCR System and QX ONE Software provide users with the capability to set up and run up to five plates concurrently through three ddPCR phases (droplet generation, thermal cycling, and droplet reading), and then analyze the results. Supported Operating System QX ONE Software is supported on the 64-bit Windows 10 operating system. BIO_CCODE00006964 The QX One System supports networking of the ddPCR system through a single company network as described below. On information and belief that the operating system may interface to and be located on a remote server.

Claims	Exemplary Disclosure of Accused Product
	 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. BIO_CC00006576
9. The method of claim 7, wherein said computer is located on a thermal cycler.	The QX ONE includes a thermal cycler and as discussed in Claims 5 and 7 (incorporated by reference), the QX ONE is also a computer. Therefore, when the claimed method is performed on the QX ONE, it is performed on a computer located on a thermal cycler. The QX One System performs the step wherein said computer is located on a thermal cycler, either literally or under the doctrine of equivalents. For example, The QX One System features a touch screen computer, which is integrated onto the instrument and has QX One Software already installed. The QX One System contains an internal thermal cycler.

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.

Claims	Exemplary Disclosure of Accused Product
	BIO_CCODE00006576 The QX One System uses thermal cycling. ▪ For the QX ONE Droplet Digital PCR System, thermal cycling is integrated into and sequentially performed by the system itself. Hence, no additional equipment or sample handling is required for this step. Refer to the QX ONE Droplet Digital PCR System and QX ONE Software User Guide (10000116512) for plate setup instructions. Use appropriate thermal cycling conditions as specified in Table 2 BIO_CCODE00007878