

USER GUIDE

CodeLink® Activated Slides

For research use only



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CodeLink® Activated Slides

Product description

CodeLink Activated slides covalently immobilize amine-modified DNA for use in microarray analysis. The slides are coated with a hydrophilic polymer containing N-hydroxysuccinimide (NHS) ester reactive groups and designed to minimize non-specific binding. DNA couples to the slide surface at pH 8-9 in a humid environment following printing. Because the NHS ester groups are moisture sensitive, printing must occur in an environment where the relative humidity is below 50%. Unused CodeLink Activated Slides must be stored in a desiccated environment.

Only one side of a CodeLink Activated slide can bind DNA. The reactive side will be up when slides are placed so that you can read “CodeLink” (Figure 1). Slide dimensions are 25 mm x 75 mm x 1 mm.

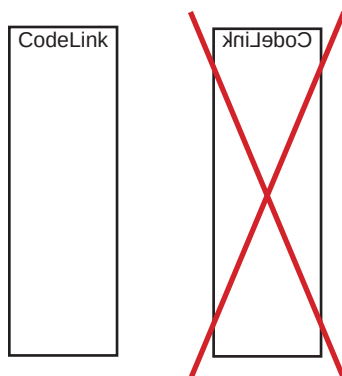


Fig 1. “CodeLink” is readable on the reactive side of the slide.

Customer support

Please contact IVDtechsupport@surmodics.com

Safety warnings and precautions

Warning: For research use only. Not recommended or intended for the diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

- Consider all chemicals as potentially hazardous. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products. Wear suitable protective clothing such as laboratory overalls, safety glasses, and gloves. Exercise caution to avoid contact with skin or eyes; if contact should occur, wash immediately with water (see Material Safety Data Sheet for specific recommendations).

Storage and handling

- Moisture sensitive. Store desiccated at room temperature.

Quality control

Each lot of CodeLink Activated Slides is tested for the ability to bind reproducibly a standard quantity of amine-modified oligonucleotides. The quantity of DNA binding is measured using fluorescently labeled control oligonucleotides, and the level of background signal is monitored in the finished product and during the manufacturing process.

Note: Within this manual, “probe” refers to the immobilized nucleic acid tethered to the slide surface and “target” refers to the free nucleic acid that is being analyzed.

Protocol

1. Materials provided by the researcher

Please refer to Appendix 1 for detailed preparation procedures.

1.1. Printing and coupling DNA probes

- 6x print buffer (300 mM sodium phosphate, pH 8.5)
- PCR purification kit or desalting columns
- Saturated NaCl humidification chamber (Add as much solid NaCl to water as needed to form a 1 cm deep slurry in the bottom of a plastic container fitted with an airtight lid. This forms a chamber with a relative humidity of approximately 75%. Alternatively, a commercially available environmental chamber can be used.)
- Slide staining racks for doing washes.

1.2. Post-coupling and hybridization

- Blocking solution (50 mM ethanolamine, 0.1 M Tris, pH 9)
- Post-coupling wash solution (4x SSC, 0.1% SDS)
- Labeled targets prepared in hybridization buffer
 - For oligonucleotide arrays (4x SSC, 0.1% SDS)
 - For PCR product arrays (4x SSC, 0.1% SDS; SSPE/formamide buffers are also effective)
- Post-hybridization wash solutions:
 - 4x SSC
 - 2x SSC, 0.1% SDS
 - 0.2x SSC
 - 0.1x SSC
- Shaker
- Microcentrifuge
- Centrifuge with microplate carriers (to spin-dry slides)
- Hotplate
- Shaking incubator
- Hybridization chambers

2. Immobilization

Preparation of amine-labeled DNA probes

2.1 Custom synthesized 3'- or 5'-amine-modified oligos.

Note: Amine contaminants (e.g., ammonia and Tris) in the DNA probe preparations will decrease the immobilization efficiency.

2.2 Amine-PCR products can be prepared by using 5'-amine-modified primer on the strand that would be complementary to the labeled target. PCR products can be purified by ethanol precipitation (perform the procedure twice), or with a commercial PCR purification kit (use 10 mM sodium phosphate buffer, pH 7.0-8.5, in the final elution step).

Note: Amine contaminants (e.g., ammonia and Tris) in the PCR product preparations will decrease the immobilization efficiency.

2.3 Store the purified DNA probes at -20°C.

Preparation of DNA printing buffer

Note: Sodium phosphate (50-150 mM, pH 8-9) works best. When contact printing, SDS added to the final concentration of 0.001% can improve reproducibility, but will slightly increase spot size.

Caution: Avoid SSC, DMSO, Tris, and betaine.

2.4 Dilute 6x print buffer 5:1 with water and oligonucleotide solution. Prepare desalted amine-oligonucleotide to a final concentration of 10-25 μ M in 1x printing buffer (50 mM sodium phosphate, pH 8.5).

2.5 For amine-labeled PCR products (0.1-1 kb), a concentration of 0.1-0.5 mg/ml DNA in 1x printing buffer is recommended.

Printing and coupling DNA

Note: Printing must occur in an environment with relative humidity <50% to retain binding activity during long print runs.

2.7 Remove the slides from the sealed package. Unused slides must be stored heat-sealed inside the foil pouch containing desiccant.

2.8 Print the DNA onto the activated slides to produce microarrays.

2.9 Place the printed slides into a slide storage box.

2.10 Set the uncovered storage box in the saturated NaCl chamber.

2.11 Seal the chamber and incubate at room temperature; overnight incubation usually produces the best results. Incubate for a minimum of four hours - maximum incubation is 72 hours.

Post-coupling processing

Note: Do not allow slides to dry prior to centrifugation (see step 2.19).

2.12 Place the slides in a slide rack and block residual reactive groups using pre-warmed blocking solution at 50°C for 30 min.

2.13 Discard the blocking solution.

2.14 Rinse the slides twice with deionized water.

2.15 Wash the slides with 4x SSC, 0.1% SDS (pre-warmed to 50°C) for 30 min on the shaker. Use at least 10 ml per

2.16 Discard the wash solution and rinse briefly with deionized water.

2.17(a) For oligonucleotide arrays, proceed to step 2.19.

2.17(b) For double-stranded DNA arrays, place the slides into boiling water for 2 minutes. Proceed to step 2.18.

2.18 Rinse the slides twice with deionized water.

2.19 Place slides in rack, and the rack into a microplate carrier.

Centrifuge at 800 rpm for 3 minutes.

2.20 Store coupled slides at ambient temperature until use. For long-term storage, maintain the slides in a desiccated environment.

3. Detection

CodeLink slides are amenable to a wide variety of hybridization assays. The procedure below is a guideline for direct expression hybridization; optimization might be required. Chambers that hold larger volumes of target solution (as compared to cover slips) produce the most reproducible data.

Hybridization

3.1 Affix the hybridization chamber to the microarray slide.

3.2 Prepare purified, labeled cDNA or oligonucleotide target in the hybridization buffer (refer to section 1.2 for the appropriate buffer).

3.3 Apply target to the microarray.

3.4 Place the slides into the shaking incubator. Shake at 300 rpm at the desired hybridization temperature.

Washing and scanning

Note: Do not allow slides to dry prior to centrifugation (step 3.7).

3.5 Remove the hybridization chamber and rinse briefly with 4x SSC.

3.6 Place slides in a rack and wash with:

- 2x SSC, 0.1% SDS at hybridization temperature for 5 min. Discard the solution. Repeat once for a total of two washes.
- 0.2x SSC at room temperature for 1 min. Discard the solution.
- 0.1x SSC at room temperature for 1 min. Discard the solution.

3.7 Spin-dry the slides (5 min at 1000 rpm).

3.8 Scan the slides.

CodeLink Appendices

Appendix 1: Stock solutions

6x Print buffer (300 mM sodium phosphate, pH 8.5).

Dissolve the following in 90 ml of nuclease-free distilled water:

0.41 g Sodium phosphate monobasic (Sigma S0751)

3.785 g Sodium phosphate dibasic (Sigma S0876)

Adjust the pH to 8.5 using 1 N NaOH or 1 N HCl. Bring the final volume to 100 ml with nuclease-free distilled water.

Blocking solution (0.1 M Tris, 50 mM ethanolamine, pH 9.0)

Dissolve the following in 900 ml of nuclease-free distilled water:

6.055 g Trizma Base (Sigma T6791)

7.88 g Trizma HCl (Sigma T6666)

3.05 g (3.0 ml) Ethanolamine (Sigma E9508)

(with stirring; mix thoroughly)

Adjust the pH to 9.0 using 6 N HCl. Bring the final volume to 1 L with nuclease-free distilled water.

10% SDS

Dissolve into 900 ml of nuclease-free distilled water:

100 g Sodium dodecyl sulfate

(also called sodium lauryl sulfate, Sigma L4522)

Heat slightly to solubilize the solid. Adjust the pH to 7.2 by adding a few drops of 6 N HCl. Bring the final volume to 1 L.

20X SSC

Dissolve into 800 ml of nuclease-free distilled water:

175.3 g NaCl

88.2 g Sodium citrate

Adjust the pH to 7.0 with a few drops of 10 N NaOH. Bring the final volume to 1 L. Sterilize by autoclaving. (Alternatively, purchase 20x SSC pre-made, Sigma S6639.)

Appendix 2: Frequently asked questions

General

Are CodeLink Activated Slides compatible with all systems?

CodeLink Activated Slides are compatible with any system that can accept a slide with the following dimensions: (width x length x thickness) 25 mm x 75 mm x 1 mm. Fluorescently labeled targets are most commonly applied, and can be monitored by scanning; radioactively labeled DNA can be quantified by phosphorimaging.

Can proteins be immobilized on CodeLink Activated Slides?

Yes. Researchers generate good results in ELISA-based formats. Protocols have not been optimized for this application and will have to be developed by the researchers.

Coating

What are the dimensions of the coated surface?

The CodeLink coating covers the entire topside of the 25 mm x 75 mm slide.

Is the coating based on silane?

The CodeLink coating is comprised of a silane base layer and a hydrophilic, amine reactive, polymeric topcoat.

What is the chemical nature of CodeLink Activated Slides?

CodeLink Activated Slides are prepared using a hydrophilic polymer containing N-hydroxysuccinimide ester groups. This polymeric coating is attached covalently to the silane basecoat.

Stability

How stable are CodeLink Activated Slides?

CodeLink Activated Slides are stable for 10 months in their original heat-sealed, desiccated packaging. Once the original packaging has been opened, any unused slides must be stored desiccated in a heat-sealed bag. A bag with a ziplock gusset alone does not provide an adequate moisture barrier to protect the activated slides.

Are CodeLink Activated Slides stable after opening the package?

CodeLink Activated Slides are stable until the expiration date (printed on the package) if they are stored desiccated in a sealed bag.

How long can the CodeLink Activated Slides be exposed to higher humidity without affecting the reactive groups?

CodeLink Activated Slides should be printed below 50% relative humidity. The recommended range is 30-45% relative humidity. At 50% relative humidity, an 8 hour printing run is possible. Use lower humidity levels for longer print times.

Are CodeLink Activated Slides stable after printing?

If CodeLink Activated Slides are stored desiccated (which also inhibits microbial contamination) the slides will be stable for an extended period. Ten-month stability has been demonstrated.

Are Link Activated Slides stable at high hybridization temperatures?

Perform long (> 24 h) hybridization experiments at or below 55°C.

Are CodeLink Activated Slides stable at high pH?

Due to the silane base coating, do not expose CodeLink Activated Slides to environments greater than pH 9.

Printing and coupling

Is it necessary to use the recommended CodeLink print buffer (50 mM sodium phosphate pH 8.5)?

The print buffer is designed specifically for use with CodeLink slides. This pH (8.5) allows maximum binding of the amine to the surface. Acceptable sodium phosphate concentrations are 50-150 mM at pH 8-9.

Is there any advantage to using highly purified oligonucleotides?

We have seen no significant advantage to further purification. The oligonucleotide must be desalted to remove competing amines, i.e., Tris.

What size spots are expected with CodeLink Activated Slides?

Due to the hydrophilic nature of the coating, the spot size may be slightly larger than that achieved with coatings that are more hydrophobic. Adding 2-8% sodium sulfate to the print buffer will decrease the spot size slightly. This addition decreases slightly the amount of DNA immobilized.

Can additives such as DMSO, betaine, or glycerol be used to prevent drying out of printed spots to facilitate the reaction?

These additives decrease binding and/or destroy spot morphology. The nature of the CodeLink Activated Slides does not require that the printed DNA spots remain solubilized for effective immobilization.

How should PCR products be purified prior to printing?

The amine primers will compete with the PCR products for binding sites on the slide surface. These primers must be removed as directed in the protocol. Do not use Tris buffers in the final elution.

Are any additional chemicals needed for coupling to occur?

No.

Why is incubation in a saturated NaCl chamber required when this step is not required for other types of slides?

Binding of amine-labeled DNA to the slide surface occurs through a thermochemical reaction. The saturated NaCl solution creates a 75% relative humidity environment that provides sufficient moisture for this reaction to proceed. If the printed slides are exposed to 100% relative humidity, the spots may enlarge or distort.

Amine modification

How is the amine moiety added to PCR products or oligonucleotides?

Oligonucleotides must be synthesized with an amine attached at either the 5'- or 3'-end. PCR products are prepared by including a 5'-amine-modified primer in the amplification reaction. Primers labeled at the 3'-end will also function in PCR, but they are more expensive to synthesize and less commonly applied. If 3'-OH is blocked, no amplification will take place through Taq polymerase.

What is the recommended spacer length?

C6 is most common and cost effective; other lengths will also work.

Is it necessary to use amine-modified DNA samples?

The best results are obtained using aminated DNA. Non-aminated DNA will provide lower levels of immobilization and hybridization signal. Non-aminated DNA binding decreases with decreasing length.

Hybridization

What is the standard sample amount applied per slide?

Target prepared from 0.5-2 µg of polyA RNA or 10-40 µg of total RNA is standard.

Can CodeLink Activated Slides be stripped and rehybridized?

CodeLink Activated Slides are not intended for rehybridization. Alkali treatment will remove the silane base coating. If desired, slides can be boiled to strip off the hybridized target.

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