SCHOTT NEXTERION® Aminosilane Coating (A+)

Protocol

Table of Contents

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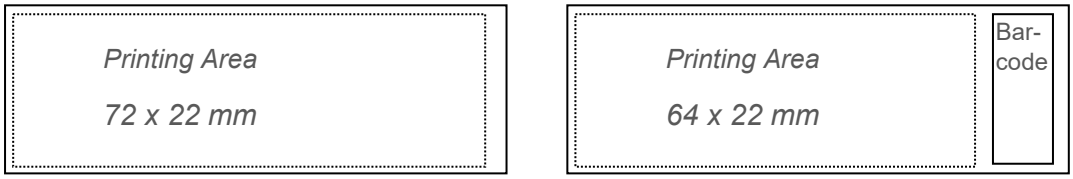
Protocol

1. Introduction

Nexterion®Slide A is manufactured using the highest quality glass (standard dimensions of 75.6 mm x 25.0 mm x 1.0 mm) and laser cutting technologies, to obtain defect and particle free substrate surfaces and excellent dimensional tolerances. The slide has a very low thickness deviation, an ultra-flat surface, and an extremely low inherent fluorescence. The coating is a multi-aminosilane, which provides an ideal surface for spotting and yields high retention of unmodified longer synthetic oligonucleotides (size ≥ 50mer), cDNA and PCR products that are readily accessible for hybridization. Stringent cleaning and chemical coating procedures favor the generation of high-quality microarrays. The density of amino-groups is uniform over the entire surface of slides and is adjusted to yield optimal binding. The spotting area is defined for an area of 72 x 22 mm for slides without barcode and 64 x 22 mm for slides with barcode.



2. Storage and handling

1. Store the packaged substrates at room temperature (20 - 25 °C) and use prior to the expiration date.
2. Open and use the substrates in a clean environment to avoid particle build-up on the printing surface.
3. Avoid direct contact with the printing surface to minimize contamination and abrasion of the coated surface.
4. Once the package has been opened, substrates should be used within 2 weeks.

3. General precautions

1. The protocols contained in this document are meant to be general guidelines only and some optimization may be required depending on the application and sample being used.
2. Refer to manufacturer supplied Material Safety Data Sheets (MSDS) for proper handling and disposal of all chemicals.
3. Nexterion® Slide A is for research use only, not for in vitro diagnostics use.

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4. Reagents required

1. Deionized water (diH_2O)
2. Dimethylsulfoxide (DMSO) - Sigma D8418.
3. Sodium dodecyl sulfate (SDS) - Fisher BP166-500.
4. Saline Sodiumcitrate (20 x SSC) - Ambion 9673.
5. Ethanol - Aldrich E7023.
6. Bovine Serum Albumin (BSA), Fraction V - Sigma A-9418.
7. Formamide - Sigma F7503.
8. Salmon Sperm DNA (Sigma D1626) or Cot1-DNA (Life Technologies 25279-011) or Poly (A)-DNA (Pharmacia 27-7836-01).

5. Equipment required

1. UV cross-linker (Stratagene Stratalinker).
2. Heat block capable of heating to 95 °C.
3. Heated water bath.
4. Cover slips (like PGC Scientific 44-596).
5. Humidified hybridization chamber (like GeneMachines HybChamber).
6. Centrifuge with slide holders or compressed nitrogen gas for drying slides.
7. Coplin jars (VWR 25457-006) or slide dish and rack combo (Fisher 900200) for washing slides.

6. Array printing

1. Mix equal amounts of oligonucleotide probe or PCR product and 50 % DMSO to obtain a minimum final probe concentration of 20 µM for oligonucleotides (size ≥ 50 mer), or 0.3 mg/ml for PCR products in 25 % DMSO (final concentration). For smaller spot sizes, 3 x SSC can be used as a printing buffer.

DNA Probes	Final Spotting Concentration
Oligonucleotides	5 - 30 µM
PCR Products	0.1 - 1 mg/ml

Spotting solutions commonly used for NEXTERION® Slide A:

Spotting Solution	Remark
50 % DMSO	larger spot size, prevents evaporation problems during long spotting runs
3 x SSC	smaller spots, standard aqueous spotting solution

2. Transfer an appropriate volume of probes to a microtiter plate.
3. Setup the arrayer according to the manufacturer's recommendations. If you were previously using slides with a thickness different from 1.0 mm, for optimal spotting you may need to re-calibrate the distance between the slide surface and the spotting pins.
4. Print the substrates at 40 – 50 % relative humidity at 20 °C to 25 °C, then allow the arrays to air-dry for 10 min.

Caution: If you use a diamond scriber to mark the boundaries of the array, this produces small glass fragments, which may get trapped under the cover slip and damage parts of the array. Carefully remove particles with a clean stream of compressed air or nitrogen before starting the print process. Alternatively, lightly mark the boundaries of the array on the backside of the slide.

7. DNA immobilisation

1. Rehydrate the printed surface for 2 - 3 sec by holding the microarray over a heated water bath (95 °C).
   Caution: Excessive rehydration may result in coalescence of the spots.
2. Snap-dry the printed array (DNA side up) by placing on a hot plate at 85 °C for 10 sec.
3. Expose the array side of the slide to UV light using a UV cross-linker at a setting of 800 mJ for oligonucleotides or 600 mJ for PCR products.
   Note: If you do not have access to a UV cross-linker, then bake the slides at 80 °C for 2 h. However, UV cross-linking is highly recommended and results in a significantly higher signal intensity compared to baking.
4. Proceed to prehybridization or store the arrays in a desiccator for no more than 2 weeks. If you want to store slides it is recommended to do this after prehybridization.

8. Array denaturation

Note: This step is only for arrays of double-stranded DNA (e.g. PCR products and genomic DNA) and is optional. Denaturation can result in higher overall signal but may also result in higher variability.

1. Immerse the arrays in 0.1 % sodium dodecyl sulphate (SDS) for 30 sec, followed by boiling water for 3 min and 70 % ethanol for 2 min.
2. Dry the arrays in an oil free air or nitrogen stream or by centrifugation (200 x g for 5 min) to avoid water stains on the slide surface.

9. Prehybridization

Prepare 100 ml of 1x prehybridization solution according to Table 1. This solution is also used for hybridization; so if you plan on not using formamide during hybridization, then do not add it into the prehybridization solution.

Note: Salmon sperm DNA can be replaced by Cot1-DNA or Poly (A)-DNA or may be excluded.

Table 1: Recipe for preparing 100 ml of 1 x prehybridization / hybridization solution

Ingredient	Amount Required	Final Concentration
20 x SSC	25 ml	5 x SSC
SDS	0.1 g	0.1 % (w/v)
BSA	1.0 g	1.0 % (w/v)
Formamide	50 ml	50 % (v/v)
Salmon Sperm DNA	10 mg	0.01 % (w/v)
diH_2O	Fill to 100 ml	

1. Immerse arrays into a container (e.g. Coplin or slide staining jar) containing pre-hybridization solution and incubate at 42 °C for 1 h in a water bath.
2. Wash the arrays by dipping five times in diH_2O.
3. Dry the arrays in an oil free air or nitrogen stream or by centrifugation (200 x g for 5 min) to avoid water stains on the slide surface.
4. Proceed to hybridization or store printed slides dry and dark for several months.

10. Hybridization

Caution: If you choose not to use formamide, make sure that your hybridization chamber seals very well and allows water to be placed inside to maintain humidity. Since the hybridization is done at a higher temperature, there is a greater chance for drying, which can lead to significant background fluorescence.

1. Re-suspend the dried, labeled sample that will be applied to the array in an appropriate amount of 1x hybridization solution as made for the prehybridization step.
   Note: If the sample is already in solution, then add appropriate amounts of each of the hybridization solution components so that the final concentrations for each is the same as listed in Table 1.
2. Denature the sample that will be applied to the array by heating it at 95 °C for 3 min in a heat block, perform a quick spin in a micro-centrifuge, then pipette the appropriate volume onto the array surface of a prehybridized slide.
   Caution: Placing the sample on ice after hybridization may cause the SDS to precipitate out of solution, which may interfere with hybridization. If the sample cannot be applied immediately after denaturation, then place it in a 42 °C heat block.
3. Carefully place a cover slip over the hybridization solution covering the array, making sure not to trap any air bubbles.
   Caution: Make sure the cover slips are appropriate for microarray use; some cover slips may require cleaning before use.
4. Transfer to a hybridization chamber containing diH_2O to maintain humidity but does not come in contact with the slide.
5. Place the sealed hybridization chamber into a water bath or incubator maintained at 42 °C if using formamide, or 50 - 65 °C if not using formamide and hybridize overnight.

11. Post-Hybridization washing

Caution: Do not allow slides to dry between washes and protect from light as much as possible.

Note: The solutions recommended below for washing are a general guideline; your application may require alternative stringency washes.

1. Remove the array from the hybridization chamber, taking care not to disturb the cover slip.
2. Place the array into a slide rack and immerse in a dish containing 2 x SSC and 0.1 % SDS. Plunge gently until the cover slip separates from the array.
3. Wash in 2 x SSC for 5 min at 42 °C, with gentle agitation.
4. Wash in 0.2 x SSC at room temperature for 1 min, plunging gently.
5. Wash in 0.05 x SSC at room temperature for 5 sec.
6. Dry the arrays in an oil free air or nitrogen stream or by centrifugation (200 x g for 5 min) to avoid water stains on the slide surface.
7. Protect the array from light, dust, and abrasion of the array surface, until ready for scanning.

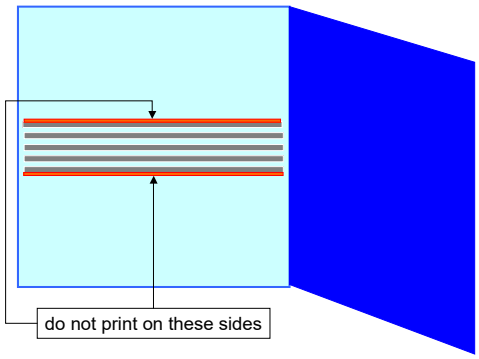
12. Appendix

Can I print on both sides of the slides?

Slide A is coated on both sides and both sides can be used as a printing side.

However, due to the possibility of outgassing of material from the slide box it is advisable to print on the side facing away from the box for the terminal slides. The drawing illustrates this in principle for slides of a 25 slide mailer box.

View of a 25 Slide Mailer



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