SCHOTT NEXTERION® Slide PLL

Protocol

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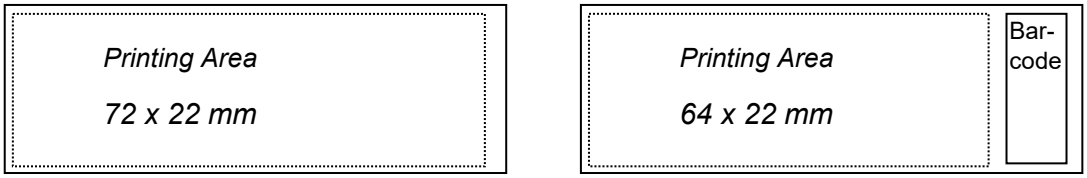
SCHOTT NEXTERION® Slide PLL

Protocol

1. Introduction

NEXTERION® Slide PLL is manufactured using the highest quality glass (standard dimensions of 75.6 mm x 25.0 mm x 1.0 mm) cut by laser 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 polymer-based coating bearing amino groups that provide an anchor surface for binding (un)modified synthetic oligonucleotides (size ≥ 50 mer), cDNA, PCR products, BACs, PACs, YACs, and cells or tissue. The density of amino groups is uniform over the entire surface of slides. The area of use (e.g. microarray printing) 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. It is also possible to store the packaged slides at 4° to 20 °C. Please let the closed package equilibrate to room temperature for 30 min before opening the pouch to prevent condensation on the coating.
2. Open the pouch and use the substrates in a clean environment to avoid particle build-up on the printing surface.
3. Avoid touching the printing surface to minimize contamination and abrasion of the coated surface.
4. Once the package has been opened, substrates should be stored in the original box at low humidity and used within 2 weeks.

3. General precautions

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

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4. Reagents

Buffers:

1. Saline sodium citrate (SSC)
2. Sodium (hydrogen) carbonate
3. Sodium phosphate (Na_2HPO_4 and/or NaH_2PO_4)
4. Phosphate buffered saline (PBS)
5. Ethylenediaminetetraacetic acid (EDTA)
6. Boric acid

Additives:

7. Dimethylsulfoxide (DMSO)
8. Sodium dodecyl sulfate (SDS)
9. Tween® 20
10. Sarkosyl
11. Triton X-100
12. Betaine
13. Sodium azide
14. Formamide
15. Dextran sulfate

Blockers:

16. Bovine Serum Albumin (BSA), Fraction V
17. Denhardt's solution
18. Salmon Sperm DNA
19. Poly(A) DNA
20. Yeast tRNA
21. Nonfat dry milk powder
22. Casein
23. Succinic anhydride
24. N-hydroxysuccinimide

Solvents:

25. Deionized water (diH_2O)
26. 1-methyl-2-pyrrolidinone
27. 1,2-dichloroethane
28. N-methylimidazole

5. Equipment required

1. Heat block capable of heating up to 95 °C
2. Heated water bath
3. Clean cover slips
4. Humidified hybridization chamber
5. Hybridization machine (optional)
6. Coplin jars or slide dish for washing slides
7. Centrifuge with slide holders or compressed oil free nitrogen gas for drying slides
8. Low volume dispensing/printing instrument (microarray spotter)
9. Detection equipment e.g. fluorescence scanner

6. Array printing

Nucleic acid probes

Mix equal amounts of oligonucleotide probe or PCR product and 2 x print buffer. We recommend 10 - 20 µM probe concentration for oligonucleotides (size ≥ 50mer), 0.3 mg/ml for PCR products, and 0.2 mg/ml for proteins/antibodies as final concentration for printing.

DNA Probes	Final print concentration
Oligonucleotides	5 – 30 µM
PCR-products	0.1 – 1 mg/ml

Protein probes

For printing proteins or antibodies, we recommend the use of PBS buffer as most proteins are stable in PBS. Proteins can usually be printed at final concentrations of 0.1 -1 mg/ml. There are several potential additives for printing listed in the table below.

In general, try to avoid glycerol concentrations > 20 % as it can affect binding of the protein to the surface. Remove high glycerol content by dialysis.

Once protein is diluted in print buffer samples should not be subjected to freeze/thaw cycles.

Protein Probes	Final print concentration
Proteins	0.1 – 1 mg/ml

Print Solution	Remark
PBS	pH 7.4, standard aqueous spotting solution for proteins

Possible additives to print buffer	Remark
Tween® 20	0.005 %, reduces protein denaturation, removal of nonspecific protein
Sarkosyl	0.001 %
SDS	0.01 %
Betaine	1.5 M, can enhance binding capacity of probe to surface (DNA), spot homogeneity
Triton X-100	0.006 %, spot homogeneity
BSA	0.1 %, spot homogeneity, can enhance antibody binding
Sodium azide	0.1 %, preservative

Printing

Transfer an appropriate volume of probes to a microtiter plate (MTP). Especially for proteins use MTP made from polypropylene.

1. Set up 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.

2. Print the substrates at 40 - 50 % relative humidity at 20 - 25 °C.

3. Immobilize the probes at 75 % relative humidity at 20 – 25 °C overnight. For sensitive proteins consider lower temperatures.

Caution: If you use a diamond scriber to mark the boundaries of the array, this produces small glass fragments, which may 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 immobilization

1. Incubate slides in a sealable container overnight at 75 % relative humidity in a dark place.

   Note: Baking slides at 80 °C for 2 h can enhance signal intensity. Exposure of the array side of the slide to UV light can also result in a higher immobilization efficiency and subsequent higher signal intensity.

2. Proceed to pre-hybridization directly or store the arrays in a desiccator for no more than 2 weeks. If you want to store the slides it is recommended to do this after pre-hybridization. For sensitive proteins consider storage at 4 °C.

8. Array denaturation

Note: This step is only needed 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 % 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. Pre-hybridization/Blocking

Nucleic acid probes

1. There are several options for pre-hybridization (= blocking): Standard blocking solutions can contain BSA or Denhardt's solution with additional blocking agents such as yeast tRNA, Poly(A) DNA or salmon sperm DNA. Chemical blocking of amino groups is possible with succinic anhydride or N-hydroxysuccinimide (NHS), although some available protocols for succinic anhydride involve hazardous solvents. Sulfo-NHS is another option for chemical blocking but much more expensive.

   Note: NHS undergoes hydrolysis under basic pH. The half-life is approximately one hour. Therefore, this reagent needs to be prepared right before use.

Possible blocking agent(s)	Blocking solution
BSA	1 % in 3 x SSC with 0.1 % SDS
Denhardt's solution	5 x in 5 x SSC with 0.1 % SDS
Succinic anhydride	70 mM in 0.1 M boric acid pH 8 with 35 % 1-methyl-2-pyrrolidinone
Succinic anhydride	1 g in 200 ml 1,2-dichloroethane + 2.5 ml N-methylimidazole
N-hydroxysuccinimide	0.1 M in 0.1 M $NaHCO_3$ pH 8

Protein probes

2. Standard blocking solutions for protein arrays are based on nonfat milk, BSA or casein dissolved in PBS. Sometimes sodium azide is added as preservative.

Possible blocking agent(s)	Blocking solution
BSA	1 - 2 % in PBS
Nonfat dry milk	3 % in PBS with 0.1 % Tween® 20
Casein	10 mg/ml in PBS

3. Immerse arrays into a container (e.g. Coplin or slide staining jar) containing pre-hybridization solution and incubate for one hour. Use elevated temperatures (e.g. 42 °C) if appropriate or lower temperatures (e.g. 4 °C) for sensitive proteins.
4. Wash the arrays by dipping five times in diH_2O.
5. 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.
6. Proceed to hybridization or store printed slides dry and dark for several months.

10. DNA 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. Resuspend the dried, labeled sample that will be applied to the array in an appropriate amount of 1 x hybridization solution.
   Note: If the sample is already in solution, then add appropriate amounts of each of the hybridization solution components.

Hybridization Solution
3 - 5 x SSC, 0.1 - 0.2 % SDS, 50 % formamide
250 mM Na_2HPO_4 pH 7.2, 4.5 % SDS, 1 mM EDTA pH 8, 1 x SSC

Potential additives	Remark
5 % dextran sulfate	decreases unspecific binding
5 % dextran sulfate	decreases unspecific binding
5 % dextran sulfate	decreases unspecific binding
Yeast tRNA	decreases unspecific binding

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 pre-hybridized 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. Make sure the water does not come into 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.
   Note: Hybridization times and temperatures can (to some extend) be adjusted for specific applications. Using automated systems for hybridization can also reduce hybridization time and ensure reproducible results.

11. (Post-Hybridization) Washing of arrays

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.

Nucleic acid probes

1. Remove the array from the hybridization chamber. (Take care not to disturb the cover slip if in use.)
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.

Protein probes

After binding of the analyte or the secondary antibody incubation or other assay components used the arrays need to be washed to remove unspecifically bound analyte/antibodies.

Standard washing solutions for proteins are mainly based on PBS with potential additional additives. We recommend PBS/Tween 20.

1. Remove the array from the reaction chamber.
2. Place the array into a slide rack and immerse in a dish containing 1 x PBS/0.1 % Tween® 20 with gentle agitation or plunging.
3. Wash at room temperature with 1 x PBS/0.05 % Tween 20 with slight agitation.
4. Wash with 1 x PBS with slight agitation.
5. Rinse with diH_2O briefly if advisable.
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?

NEXTERION® Slide PLL 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.