SCHOTT NEXTERION® Slide AL

Protocol

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1. DNA application

1.1 Introduction

Nexterion® Slide AL 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 slide surfaces and excellent dimensional tolerances. The slide has a very low thickness deviation, an ultra-flat surface, and an extremely low inherent fluorescence. The aldehyde surface coating allows for the efficient covalent and directed binding of amino-modified oligonucleotides and/or PCR-products. Amino-functionalities of nucleic acids react with the aldehyde modified glass surface to form a covalent bond via the Schiff's base aldehyde-amine chemistry. The density of aldehyde groups is uniform over the entire surface of slides and is adjusted to yield optimal binding. The chemically reactive and homogeneous 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.

1.2 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 is opened, substrates should be used within 8 weeks if stored under inert condition inside a desiccator and protected from light at room temperature.

1.3 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 and Data Sheets (MSDS) for proper handling and disposal of all chemicals needed.
3. NEXTERION® Slide AL is for research use only, not for in vitro diagnostic use.

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1.4 Reagents required

1. Deionized water (diH_2O)
2. 2 x NEXTERION® Spot Solution or 3 x SSC or 3 x SSC containing 1.5 M betaine (final concentration)
3. Hybridization Buffer NEXTERION® Hyb (formamide-free) or 3 - 5 x SSC containing 0.1 % SDS with or without competitor DNA and formamide
4. Saline Sodium Citrate (20 x SSC) DMSO is not recommended as a co-spotting reagent
5. Sodium Dodecyl Sulfate (SDS)
6. Aldehyde Blocking Solution (Dissolve 1.0 g $NaBH_4$ in 300 ml PBS and 100 ml ethanol. Ethanol is used to reduce bubbling. Prepare 30 min ahead of use and do not reuse solution).

1.5 Equipment required

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

1.6 Array printing

1. Mix equal amounts of oligonucleotide probe or PCR-product and 2 x NEXTERION® Spot to obtain a recommended final probe concentration according to the following table:

DNA Probes	Final spotting concentration
Oligonucleotides	10 – 20 µM
PCR-products	0.1 – 1 µg/µl

Notes:

a) Use of the NEXTERION® Spot Solution is recommended especially when spotting oligonucleotides.

b) For Ring-And-Pin systems and for non-contact printing systems, a lower concentration of NEXTERION® Spot could be tried.

c) To increase the spot sizes, detergents (Cetyl-trimethylammoniumbromid, Triton® X-100, Sarkosyl, Tween® 20 or SDS) may be added to NEXTERION® Spot Solution, with a final concentration between 0.01 and 0.1 % in the 2x solution.

d) Alternatively, 3 x SSC or 3 x SSC containing 1.5 M betaine (final concentration) can be used as spotting buffers.

e) Do not use any spotting solution containing primary amino-groups like Tris.

f) PCR-products amplified with amino-modified primers are preferred for spotting. However unmodified PCR-products can be also immobilized, because of their exocyclic amino-groups.

g) When an amino-functional primer is used to generate the PCR-products, the unused primers should be separated from the PCR-products using a suitable method prior to spotting.

h) Amino-modified oligonucleotides are immobilized more efficiently than unmodified oligonucleotides.

2. Transfer an appropriate volume of probes to a microtiter plate.

   Note: DNA-probes in Nexterion® Spot can be stored at -20 °C until ready for spotting. If the probe solution shows a white precipitation prior to spotting, heat the probes to 50 °C to 80 °C for 2 min, avoiding any change of concentration by condensation.

3. Setup the arrayer according to the manufacturer's recommendations. If you were previously using slides that were thicker than 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 to 25 °C.

   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.

1.7 DNA immobilization

1. Incubate printed microarray slides in humidity chamber (> 90 % relative humidity) at room temperature for 15 min for completion of the covalent binding of DNA-probes on the slide surface after spotting.
2. Incubate microarray slides at 120 °C for 60 to 90 min.
3. Proceed to washing.

   Note: After spotting and immobilization, the arrays can be used immediately or stored under dry and dark conditions at room temperature. The washing steps after immobilization should not be carried out until immediately prior to hybridization.

1.8 Washing

1. Wash slides to remove unbound probe molecules and buffer substances to avoid interference with subsequent hybridization experiments.
   a. Rinse 2 x 2 min in 0.2 % SDS at room temperature.
   b. Rinse 2 x 2 min in diH_2O at room temperature.
   c. <optional>Denaturation step for arrays spotted with PCR-probes: 1 x 3 min in boiling diH_2O.
   d. Rinse 1 x 1 min in diH2O at room temperature.

   Note: The volume of washing solution should be at least 250 ml for 5 slides.

2. Proceed to Blocking immediately.

   Note: Make sure that slides do not dry in between washing steps and between washing and blocking.

1.9 Blocking

1. Block the slides with Aldehyde Blocking Solution as follows:
   a. Incubate slides 1 x 15 min in Aldehyde Blocking Solution at room temperature. The volume of blocking solution should be at least 100 ml for 5 slides.

   b. Rinse 2 x 2 min in 0.2 % SDS at room temperature.

   c. Rinse 2 x 2 min in diH_2O at room temperature.

2. Dry the slides in an oil-free air or nitrogen stream or by centrifugation (200 x g for 5 min) to avoid any water stains on the slide surface.
3. Proceed to hybridization.

1.10 Hybridization

1. Re-suspend the dried, labeled target to be applied to the array in Nexterion® Hyb. In case the target is already dissolved in a different buffer or in water, the sample can also be diluted in NEXTERION® Hyb to get at least 90 % (v/v) buffer in the final hybridization solution.

   Note:

   a) The amount of buffer depends on the desired target concentration and the size of hybridization coverslip used.

   b) As an alternative to the NEXTERION® Hyb, a buffer with 3 - 5 x SSC containing 0.1% SDS can be used.

   c) The length of hybridization time and the hybridization temperature depend on target concentration, sequence, length, etc. and need to be optimized for each special application (i.e. 16 h 42 °C when using formamide containing hybridization buffer and 16 h 65 °C when using formamide free hybridization buffer).

2. Denature the suspended target by heating at 95 °C for 3 min in a water-filled well of a heat block, perform a quick spin in a micro-centrifuge, then pipette the appropriate volume onto the array surface of a blocked slide under the coverslip or inside a hybridization station.

   Caution: If the sample cannot be applied immediately after denaturation, then place it in a 42 °C water-filled well of a heat block.

1.11 Post-Hybridization washing

Caution: Do not allow slides to dry in between washes and protect from light as much as possible. Never wash the slides with diH_2O after hybridization.

Note: The solutions recommended below for washing are a general guideline; your application may require alternative washing stringencies. The volume of the washing solution should be at least 250 ml for 5 Slides.

1. Place the array into a slide rack and immerse in a dish containing 2 x SSC and 0.2 % SDS. Wash in the above solution 1 x 10 min at room temperature.
2. Wash 1 x 10 min in 2 x SSC.
3. Wash 1 x 10 min in 0.2 x SSC at room temperature.
4. Dry the array 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.
5. Protect the array from light, dust and abrasion of the array surface, until ready for scanning. Ensure that the laser and filter set of the scanner is compatible with the fluorescent labeling of the probe molecules.

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2. Protein application

2.1 Introduction

Technical Instructions for Spotting Protein Microarrays

Product overview

NEXTERION® Slide AL 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 slide surfaces and excellent dimensional tolerances. The slide has a very low thickness deviation, an ultra-flat surface, and an extremely low inherent fluorescence. NEXTERION® Slide AL was developed to provide the opportunity for fast and efficient coupling of proteins onto activated glass slides. The aldehyde surface coating reacts with primary amino groups provided by amino acid side chains (i.e. of lysine) immediately to form covalent bonds. Stringent cleaning and chemical coating procedures favor the generation of high-quality microarrays. The density of aldehyde 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.

2.2 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 is opened, substrates should be used within 8 weeks if stored under inert condition inside a desiccator and protected from light at room temperature.

2.3 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 and Data Sheets (MSDS) for proper handling and disposal of all chemicals.
3. NEXTERION® Slide AL is for research use only, not for in vitro diagnostic use.

2.4 Reagent required

1. Protein Print Buffer: PBS (137 mM NaCl, 9 mM KOH, 11.3 mM NaH_2PO_4) pH 7.0
2. Blocking Buffer: 1 % BSA in PBST (Alternatively, 3 % nonfat milk in PBST can be used)
3. Wash Buffer: PBST (PBS with 0.05 % v/v Tween® 20 pH 7.4)
4. Rinse Solution: PBST

2.5 Equipment required

1. Humidified hybridization chamber (like GeneMachines HybChamber) or place a 1-inch layer of NaCl in a chamber filled with water and cover with an airtight lid. This forms a chamber with a nominally 75 % relative humidity.
2. Centrifuge with slide holders or compressed nitrogen gas for drying slides.
3. Cover slips (like PGC Scientific 44-596).
4. Coplin jars (VWR 25457-006) or slide dish and rack combo (Fisher 900200) for washing slides.

2.6 Protein immobilization

Print proteins at 50 % relative humidity and then place arrays in a humidity chamber at room temperature 1 hour.

2.7 Array printing

Print proteins at a final concentration of 0.05 - 0.2 mg/ml in the print buffer.

NEXTERION® Slide AL is compatible with all microarray printing or spotting methods, including contact printing and piezo or ink-jet technologies.

2.8 Blocking

Block slides for one hour in blocking solution at room temperature with slow shaking.

2.9 Target incubation

1. Dilute the labeled target sample in an appropriate volume of incubation or blocking buffer to cover the whole array.
2. Incubate with target solution for 1 hour.
3. Remove the coverslip and place the arrays into a slide rack. Immerse in a dish containing PBST. Wash with shaking for 10 minutes. Repeat.
4. Wash in PBS for 10 minutes with agitation.
5. Dry the array 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. Protect the array from light, dust, and abrasion of the array surface, until ready for scanning.

2.10 Assay conditions

For protein incubation steps on the printed slide, we have found that PBST works well as a dilution buffer for the target solutions. The blocking buffer can also be used if an increase in nonspecific binding is observed.