Protocol

SCHOTT

Nexterion® Slide A+
DNA application

Dok-Nr.:	LS6-HCF-S-002
Version:	1.0
Seite:	1/7
Datum:	© September 2006

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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 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 amino surface coating allows for the efficient covalent binding of molecules, such as PCR-products, cDNA molecules and longer, synthetic oligonucleotides (size ≥ 50 mer).
Nexterion® Slide A+ is compatible with most common printing, slide processing and hybridization protocols for aminosilane slides, giving users the opportunity to employ their established microarray processes. It is an ideal substrate, which allows scientists to change PCR product and/or cDNA probes to longer oligonucleotide probes without changing the surface chemistry and protocol. A re-optimization of the entire process for the oligonucleotide applications is not necessary. Thus Nexterion Slide A+ allows an economic transfer not only from PCR products and/or cDNA molecules to longer oligonucleotides, but also from any aminosilane slide to Nexterion® Slide A+.
The immobilization of DNA probes to Nexterion® Slide A+ is achieved in a two-step process. First, the negatively charged DNA probes form ionic bonds with the positively charged surface of Nexterion® Slide A+. A non-directed, irreversible immobilization is then achieved by UV-cross-linking. Amino-modification of the nucleic acids is not required, but such modification will not interfere with immobilization.
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 is opened, substrates should be used within 8 weeks if stored under inert condition inside a desiccator and protected from light at room temperature.

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 A+ is for research use only, not for in vitro diagnostic use.

4 Reagents required

1. Deionized water (diH_2O).
2. 2x Nexterion® Spot Solution or 3x SSC, 3x SSC containing 1.5M betaine or 50% DMSO.
3. Hybridization Buffer Nexterion® Hyb (formamide-free) or 3-5x SSC + 0.1% SDS with or without competitor DNA and formamide.
4. Saline sodium citrate (20x SSC)- Ambion 9673.
5. Sodium dodecyl sulfate (SDS)- Fisher BP166-500 or 10% SDS solution for washing (10 g sodium dodecyl sulfate in 100 ml diH_2O, dissolve at room temperature).
6. 0.1% SDS (10ml 10 % SDS solution in 1000 ml diH_2O).
7. Amino Blocking Solution (5 g succinic anhydride + 315 ml n-methylpyrrolidone + 35 ml 0.2 M sodium-borate pH 8. Add sodium-borate freshly before use).
8. Pre-Hybridization Buffer (3-5x SSC, containing 0.1% SDS and 0.1 mg/ml BSA) or alternatively 25 ml Nexterion Hyb® + 25 ml diH_2O+ 500 mg BSA (volume for 5 slides).

5 Equipment required

1. UV cross-linker (Stratagene Stratalinker) or, alternatively, an oven.
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. Dissolve oligonucleotide probe or PCR product in the appropriate spotting solution to obtain the recommended final probe concentration:

DNA Probes	Final Spotting Concentration
Oligonucleotides	**2 - 20 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
3x SSC	smaller spots, standard aqueous spotting solution
3x SSC containing 1.5 M betaine	larger spots, prevents evaporation problems during long spotting runs, very homogeneous spots
Nexterion® Spot	smaller spots, phosphate buffer based solution

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

   Note: DNA-probes in Nexterion spotting solution can be stored at -20°C until spotting. If the probe solution shows a white precipitation prior to spotting, heat the probes to 50 to 80°C for 2 min and avoid 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 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.

7 DNA immobilization

1. For covalent binding of DNA-probes on the slide surface after spotting it is necessary to UV-cross link the slides at 250 mJ. Alternatively the DNA can be covalently immobilized by incubating the slides at 80°C for 2 hours.
2. Incubate the slides at room temperature for 12 h (e.g. store slides over night).
3. Proceed to Washing

> **Note**: After spotting and immobilization, the arrays can be used immediately or stored under dry, dark conditions at room temperature for several month. The washing steps after immobilization should be carried out immediately before hybridization.

8 Washing and blocking

After immobilization it is important to remove unbound DNA-molecules and buffer substances from the slides by extensive washing to avoid any interference with subsequent hybridization experiments. To avoid bleeding of the spots it is important to perform the washing steps very quickly by moving the slides (slide holder) up and down in the rinsing solution rather then using a shaker. The blocking is either done by prehybridization with BSA or by reaction of the amino-groups with succinic anhydride.

Protocol with prehybridization, duration 50 min:

1. 1 x 10 to 20 sec in 0.1% SDS at room temperature
2. 1 x 10 to 20 sec in diH_2O at room temperature
3. (Denaturing step for arrays spotted with PCR-probes)

1 x 3 min in boiling diH_2O

4. 1 x 45 min in prehybridization buffer at 42°C
5. 1 x 10 to 20 sec in diH_2O at room temperature
6. Dry the arrays 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.

Alternatively:
Protocol with chemical blocking, duration 20 min:

1. 1 x 15 min in Amino Blocking Solution at room temperature
2. 1 x 10 to 20 sec in 0.1% SDS at room temperature
3. 1 x 10 to 20 sec in diH_2O at room temperature
4. (Denaturing step for arrays spotted with PCR-probes)

1 x 3 min in boiling 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 any water stains on the slide surface.

9 Hybridization

1. Re-suspend or dilute the labeled target in Nexterion Hybridization Buffer (alternatively 3-5 x SSC containing 0.1% SDS) 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 chamber used.
   b) As an alternative a buffer with 3–5x SSC + 0.1% SDS with or without competitor DNA and formamide 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 heat block, perform a quick spin in a microcentrifuge, 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 heat block. Work rapidly when adding the target; use of filter tips is recommended.

10 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 2x SSC and 0.2% SDS. Wash in the above solution 1 x 10 min at room temperature.
2. Wash 1 x 10 min in 2x SSC.
3. Wash 1 x 10 min in 0.2x 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.

11 Important information about patents

Using arrays based on SCHOTT Nexterion products for dual color analysis on a single array in which at least two different samples are labeled with at least two different labels may require a license under one of the following patents: U.S. patent nos. 5,770,358 or 5,800,992 or 6,225,625 and U.S. patent no. 5,830,645. Manufacturing and use of probe arrays may require a license under the following patents: U.S. patent no. 6,040,138 or 5,445,934 or 5,744,305 and under the following patents owned by Oxford Gene Technology Ltd. („OGT“): European patent no. EP 0,373,203, U.S. patent nos. 5,700,637 and 6,054,270 and Japanese patent nos. 3393528 and 3386391 ("The OGT patents"). Other patents may apply. The purchase of Nexterion® products does not convey any license under any of the OGT patents or any of the other patents referred to. For all applications SCHOTT North America Inc. and SCHOTT Jena[er] Glas GmbH make no representation or warranty that the practice of its technology and products or any improvement will not infringe or violate any domestic or foreign patent of any third party. Before making or using any oligonucleotide arrays you should contact OGT to discuss a licence. To inquire about licensing under the OGT patents, please contact OGT at licensing@ogt.co.uk.