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<tr><td colspan="2">Protocol</td><td colspan="2"> </td></tr>
<tr><td colspan="2" rowspan="4">Nexterion® Slide AStar
DNA application</td><td>Dok-Nr.:</td><td>LS6-HBM-M-002</td></tr>
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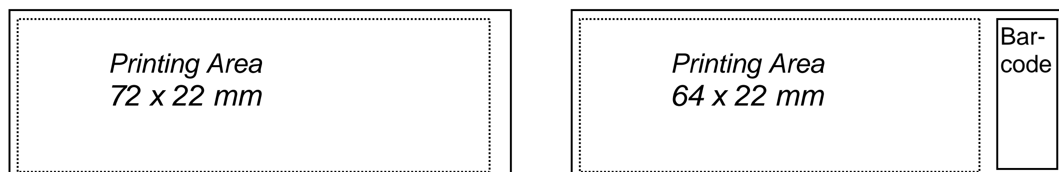
1 Introduction

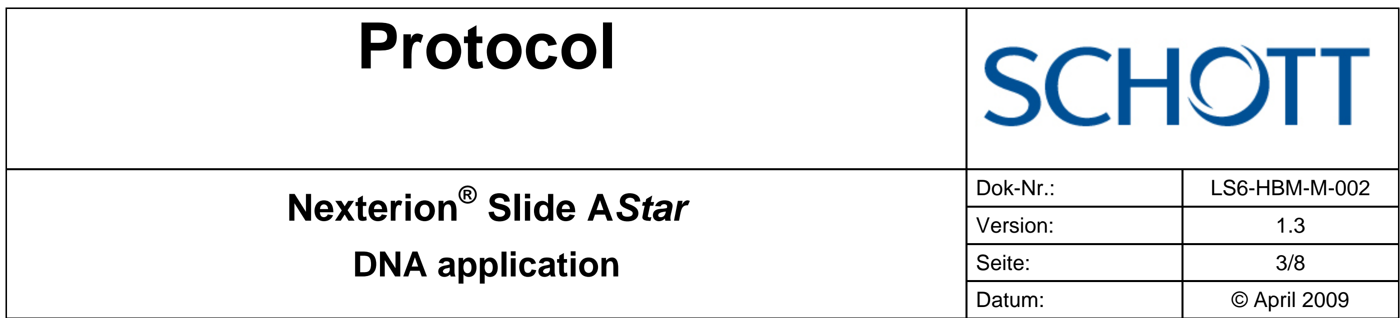
Nexterion® Slide A*Star* is manufactured using the highest quality glass (standard dimensions of 75.6 mm x 25.0 mm x 1.0 mm) and laser cutting technologies, to obtain defect and particle free slide surfaces and excellent dimensional tolerances. The slide has a very low thickness deviation, an ultra-flat 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 synthetic oligonucleotides.
Nexterion® Slide A*Star* 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 works with almost all common aminosilane protocols and DNA print buffers suitable for aminosilane substrates as 50 % DMSO, Pronto!™ Universal, Nexterion® Spot AHD, or phosphate based buffers. The combination of high-density attachment with a very low background results in superior signal-to-noise ratios in microarray experiments. Both surfaces of the slide have been coated. The immobilization of DNA probes to Nexterion® Slide A*Star* 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*Star*. A non-directed, irreversible immobilization is then achieved by baking at 80 °C for 2 h or UV crosslinking. 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*Star* is for research use only, not for in vitro diagnostic use.

4 Reagents required

1. Deionized water (diH_2O).
2. 2 x Nexterion® Spot Solution, Nexterion® Spot AHD or 50% DMSO.
3. Nexterion® Block A.
4. Hybridization Buffer: Nexterion® Oligo Hyb (contains formamide).
5. Saline sodium citrate (20 x SSC) - Ambion 9673.
6. 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).
7. 0.1 % SDS (10 ml 10 % SDS solution in 1000 ml diH_2O).

5 Equipment required

1. Oven, or alternatively, an 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 blow off with 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 - 10 µM
PCR Products	0.1 – 1 mg/ml

Spotting solutions commonly used for Nexterion® Slide A*Star*:

Spotting Solution	Remark
50 % DMSO	larger spot size, prevents evaporation problems during long spotting runs
Nexterion® Spot	smaller spots, standard aqueous spotting solution
Nexterion® Spot AHD	small spots, excellent spot morphology, low evaporation buffer

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 - 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 - 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. Incubate printed slides for 16 - 24 h (e.g. dessicator).
2. For covalent binding of DNA-probes on the slide surface DNA can be immobilized by incubating the slides at 80 °C for 2 hours in an oven. Make sure that your oven is clean to avoid contamination of the slides. Alternatively the DNA can be UV-cross linked at 500 mJ.
3. Proceed to blocking.

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

8 Blocking

After immobilization it is important to remove unbound DNA-molecules and buffer substances from the slides by washing to avoid any interference with subsequent hybridization experiments. To avoid bleeding or comet tailing of the spots, it is important to perform the blocking step.

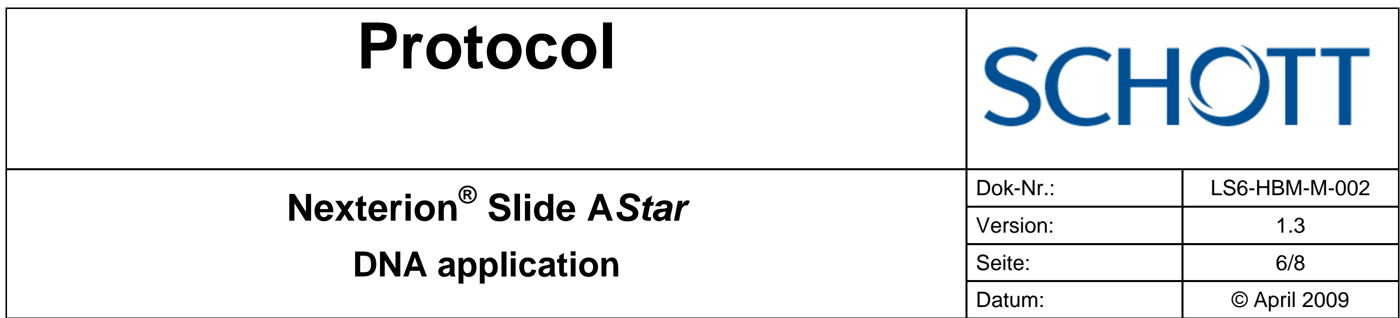
1. 1 x 45 min in Nexterion® Block A at room temperature (low agitation).
2. 1 x 10 to 20 sec in fresh Nexterion® Block A solution at room temperature
3. Dry the arrays in an oil-free air or nitrogen stream or by centrifugation (200 x g for 5 min) to avoid any water stains on the slide surface.

9 Hybridization

1. Re-suspend or dilute the labeled target in Nexterion® Oligo Hyb Buffer to get at least 95 % (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) The temperature of hybridization should be 42 °C.
   c) The length of hybridization time depend on target concentration, sequence, length, etc. and need to be optimized for each special application (i.e. 2 - 16h).

   Caution: If a hybridization station is used, use the low agitation mode, as too strong agitation might result in signal loss.



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 clean 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 and avoid bubble formation below the coverslip.

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.

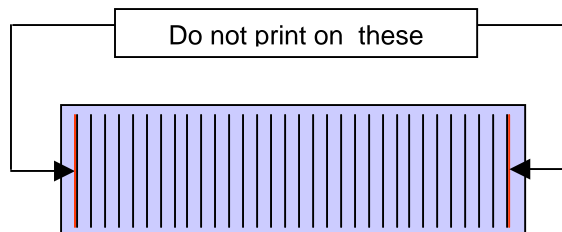
1. Place the array into a slide rack and immerse in a dish containing 2 x SSC and 0.2 % SDS. Wash in the above solution 1 x 10 min at room temperature.
2. Wash 1 x 10 min in 2 x SSC.
3. Wash 1 x 10 min in 0.2 x SSC at room temperature.
4. Dry the array in an oil free air or nitrogen stream or by centrifugation (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.

11 Appendix

Can I print on both sides of the slides?

Slide A*Star* 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 wall. The drawing illustrates this in principle for slides of a 30 slide mailer box.

View of a 30 slide mailer



12 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 SCHOTT 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 Technical Glass Solutions 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.