Protocol

Nexterion® Slide A*Star* MPX 16 **DNA-application**	Dok-Nr.:	LS6-HBM-M-002
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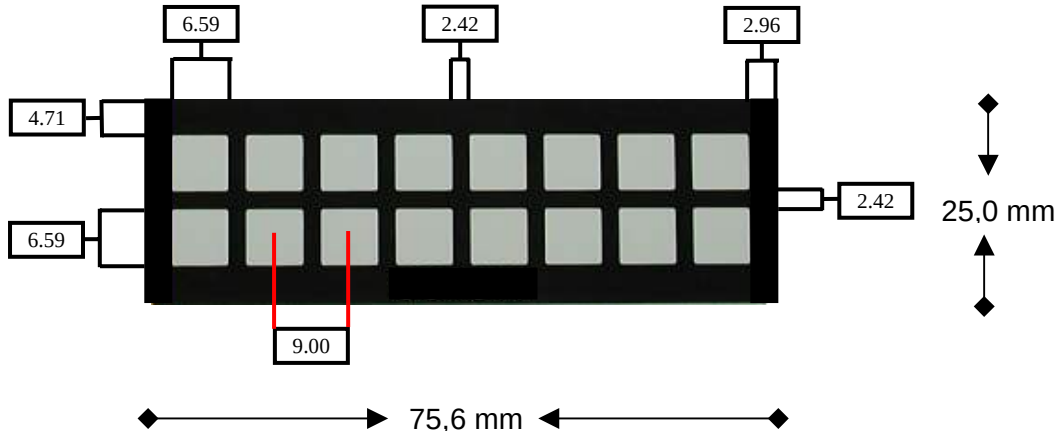
1 Introduction

Nexterion® Slide A*Star* MPX 16 is developed for fast and efficient immobilization of DNA molecules onto activated glass slides that permit superior reproducibility of microarray data and higher sensitivity. The slide 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. Nexterion® Slide A*Star* MPX 16 is cleaned and coated in cleanroom atmosphere and a strict process control in all fabrication steps ensures excellent coating uniformity and batch-to-batch reproducibility.

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, Next 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.

The uniform and ultrahydrophobic patterning material separates the slide surface into 16 individually addressable subarray chambers allowing multiplexed microarray analysis. Nexterion® Slide A*Star* MPX 16 is delivered together with superstructures and sealing strips to support excellent sample mixing and to minimize cross contamination as well as sample evaporation during the hybridization step.

Additionally re-usable microtiterplate-size-trays for automated high-throughput processing of Nexterion® Slide AStar MPX 16 are available separately. Each MPX-4 tray can hold up to four slides.



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.
5. Only one side of each slide is patterned and must therefore be used (readable "SCHOTT Nexterion"). This inscription can also be used for the well orientation

3 General precautions

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.
Refer to manufacturer supplied Material Safety and Data Sheets (MSDS) for proper handling and disposal of all chemicals.
Nexterion® Slide A*Star* MPX 16 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. UV cross-linker (Stratagene Stratalinker).
2. Heat block capable of heating to 95°C.
3. Heated water bath.
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.

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 Printing guidelines

Nexterion® Slide A*Star* MPX 16 is compatible with all microarray printing or spotting methods, including contact and non-contact printing technologies.
The following table displays the maximum number of probes per subarray well for Nexterion® Slide A*Star* MPX 16 based on theoretical calculations using pitch and type of source plate. The color-coding in the table indicates, for each pin configuration, the number of pins used for intra-well printing.

Detailed printing guidelines are available online at:
www.us.schott.com/nexterion

Maximum Probe Densities for Nexterion Slide MPX 16

Pitch (microns)	96 Well Sourceplate	384 Well Sourceplate					
Pin Configurations	*(1 x 1-2-4), (2 x 1-2-4)*	*(1 x 1)*	*(1 x 2-4-8)*	*(2 x 1)*	*(2 x 2-4-8)*	*(4 x 1)*	*(4 x 2-4-8)*
100	3364	3364	1508	1508	676	1508	676
150	1444	1444	608	608	256	608	256
200	784	784	336	336	144	336	144
250	484	484	220	220	100	220	100
300	324	324	108	108	36	108	36
350	256	256	96	96	36	96	36
400	169	169	52	52	16	52	16
450	144	144	48	48	16	48	16
500	100	100	20	20	4	20	4

1 Pin / MPX well
2 Pins / MPX well
4 Pins / MPX well

Security spacing is 300µm + pitch from well borders.

8 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.

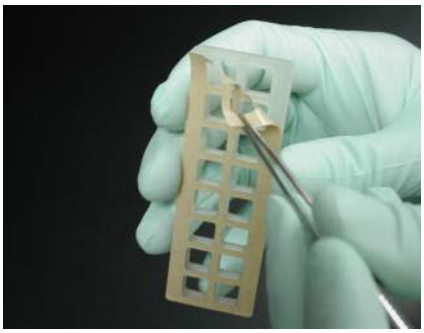
9 Blocking

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.

10 Hybridization

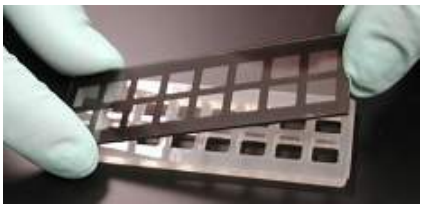
1. Remove the protective back covering from the superstructure by hand or by using a forceps.



2. Apply the superstructure on the slide a) from top or b) by inverting the slide on the superstructure, followed by c) firmly securing the superstructure on the slide.

a) Application from top

b) Application in inverted position



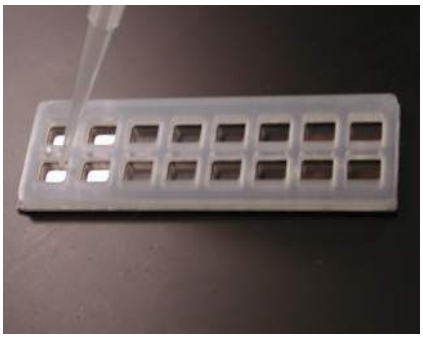
c) Securing the superstructure

3. 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) At least 7.5 pmol of each target in 30µl of hybridization buffer per well is recommended.
   b) The amount of buffer depends on the desired target concentration and the size of hybridization chamber used.
   c) The temperature of hybridization should be 42 °C.
   d) 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).

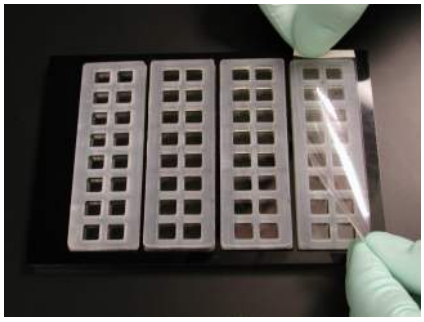
   Caution: If a hybridization station is used, use the low agitation mode, as too strong agitation might result in signal loss.

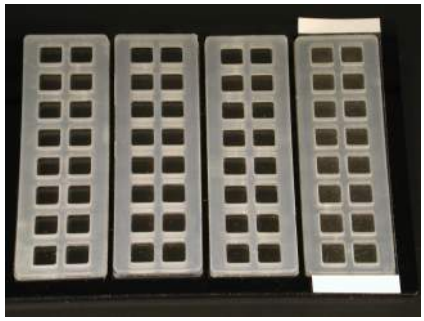
a) Addition of target to a single slide



b) Addition of target to multiple slides in a tray

4. Seal the wells immediately with the supplied sealing strips, as shown below, ensuring that the seal makes good contact with the superstructure.





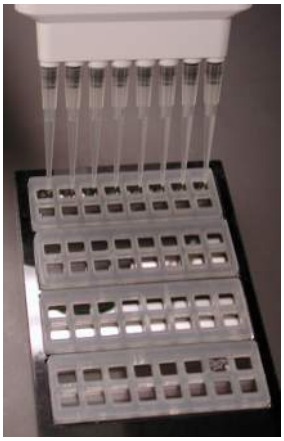
11 Post-Hybridization washing

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

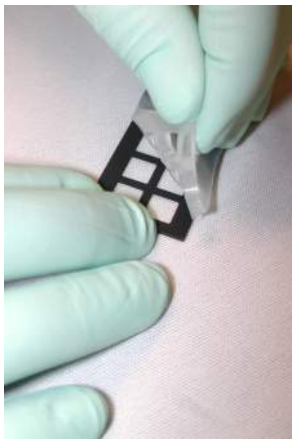
Note: The solutions recommended below for washing are a general guideline; your application may require alternative stringency washes.

1. Aspirate the target from the wells using a multi-channel pipette and immediately fill the wells with 100 µl of 2x SSC. Aspirate the wash solution and fill the wells with fresh 2x SSC. Repeat this procedure 3 times.
2. Remove the superstructure immediately and place the slides into a slide rack.

a) Aspiration using a multi-channel



b) Removal of superstructure



3. Immerse the slide rack in a dish containing 2x SSC and 0.2% SDS. Wash in the above solution 1 x 10 min at room temperature.
4. Wash 1 x 10 min in 2x SSC.
5. Wash 1 x 10 min in 0.2x SSC at room temperature.

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

6. 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.
7. Protect the array from light, dust and abrasion of the array surface, until ready for scanning.

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.