Protocol

NEXTERION® Slide H MPX 16 **DNA-application**	Dok-Nr.:	LS6-HBM-M-002
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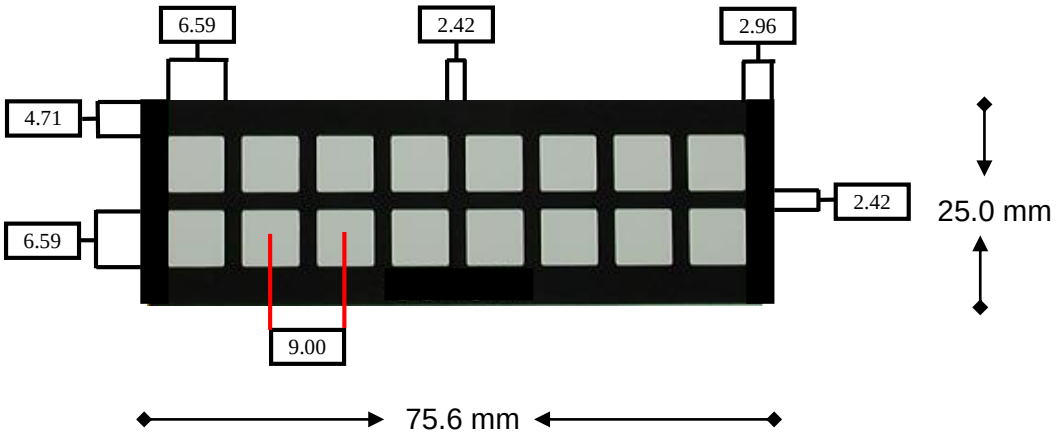
Store at –20 °C prior to use. Allow package to equilibrate at room temperature before opening.

1 Product overview

NEXTERION® Slide H 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 H 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 H MPX 16 is especially suited for the immobilization of oligonucleotides. The multi-component organic hydrogel coating provides high probe binding capacity, while the uniquely designed coating matrix inhibits non-specific binding. The three-dimensional hydrogel with amine-reactive groups allows efficient end attachment of amine-modified oligonucleotides for optimal orientation during hybridization. The combination of high-density specific attachment with a low-background matrix 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 H 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 H MPX 16 are available separately. Each MPX-4 tray can hold up to four slides.



2 Storage and handling

1. The reactive groups on the NEXTERION® Slide H coating will undergo hydrolysis reactions if not properly protected from moisture. The slides are packaged in moisture barrier bags for shipment and storage. It is strongly recommended to store the slides at -20 °C in their original packaging prior to use, as the hydrolysis of NEXTERION® Slide H coating is extremely slow at low temperature. The shelf life is 6 months when stored at -20 °C.

2. The packaging should be allowed to equilibrate completely at room temperature prior to opening. Failure to do so will lead to condensation on the slide surface and loss of activity. After opening, seal any unused slides in the reusable pouch with desiccant and re-freeze.
3. Avoid direct contact with the surface of the slides to minimize contamination and abrasion of the coated surface. Always wear gloves and hold slide edge.
4. NEXTERION® Slide H should be opened in a clean environment to avoid the build-up of particulate debris on the coated surface.
5. Only one side of each slide is patterned and must therefore be used (readable "SCHOTT NEXTERION"). This inscription can also be used for well orientation.

3 General precautions

1. The protocols contained in this document are meant to be general guidelines, and some optimization may be required depending on the specific 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 Slide H MPX 16 is for research use only, not for in vitro diagnostic use.

4 Reagents required

1. When printing at 50 % relative humidity, the following buffer should be utilized: 300 mM sodium phosphate (pH 8.5) containing 0.005 % Tween® 20 and 0.001 % sarkosyl.
2. When printing at 30 % relative humidity (suggested for long print runs), the following buffer should be utilized: 150 mM sodium phosphate (pH 8.5) containing 0.001 % Tween® 20.
3. Print buffers should be prepared immediately before printing.
4. With most pin printers, the Tween® 20 concentration can be adjusted to tune the spot size; less Tween® 20 yields smaller spots and vice versa.
5. DMSO is not recommended as a co-spotting reagent

6. Blocking Solution: 100 mM phosphate buffer, 25 mM ethanol amine, 0.01 % Tween® 20, pH 8.5
   Preparation:
   → stock solutions needed
   100 mM Na_2HPO_4 with 25 mM ethanolamine
   - dissolve 4.26 g Na_2HPO_4 in 300 ml diH_2O
   - add 0.45 ml of 100 % ethanolamine

   → pH 10.7 (24 °C)
   100 mM $NaH_2PO_4 \cdot 2\ H_2O$ with 25 mM ethanolamine
   - dissolve 15.6 g $NaH_2PO_4 \cdot 2\ H_2O$ in 1000 ml diH_2O
   - add 1.50 ml of 100 % ethanolamine

   → pH 5.6 (24 °C)
   10 % Tween® 20 solution
   - 10 ml of 100% Tween® 20 + 90 ml diH_2O

   → pH adjustment
   - provide 300 ml of 100 mM Na_2HPO_4 with 25 mM ethanolamine
   - add slowly about 250 ml of 100 mM $NaH_2PO_4 \cdot 2\ H_2O$ with 25 mM ethanolamine until pH 8.5 is reached
7. finally add appropriate volume of 10 % Tween® 20 (0.55 ml for 550 ml solution)
8. 1x Hybridization Buffer: 2 x SSC containing 0.1 % SDS and 0.1 % salmon sperm DNA (formamide can be added if required)
9. Use of desalted 3' or 5' amine-modified oligonucleotides of highest purity are recommended.

5 Equipment required

1. Heat block capable of heating to 95 °C.
2. Heated water bath.
3. Humidified hybridization chamber (like GeneMachines HybChamber) or place a 25 mm (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.
4. Centrifuge with slide holders or compressed nitrogen gas for drying slides.
5. Coplin jars (VWR 25457-006) or slide dish and rack combo (Fisher 900200) for washing slides.

6 Array printing

Synthesized 3' or 5' amino-modified oligonucleotides should be desalted and purified preferably to HPLC grade to ensure that residual nucleophiles such as Tris (hydroxymethyl)-aminomethane (Tris), ethanol amine, or free ammonium ions do not inhibit coupling efficiency. *Do not dilute oligonucleotides in Tris or any other amine-containing buffer*.

1. Dilute the amino-modified oligonucleotides to a final spotting concentration of 20 µM in the printing buffer.

2. Transfer an appropriate volume of probes to a microtiter plate.
3. Setup the arrayer according to the manufacturers 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 oligonucleotides at 30 % to 50 % relative humidity. 30 % humidity is optimal for longer print runs.

Post-print incubation: For best oligonucleotide coupling, place the printed arrays in a chamber maintained at 75 % relative humidity for two hours, followed by storage in a vacuum desiccator for at least two hours to overnight.

7 Printing guidelines

NEXTERION® Slide H 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 H 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.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 buffer is 300 microns + pitch from well borders.

8 Storage of printed slides

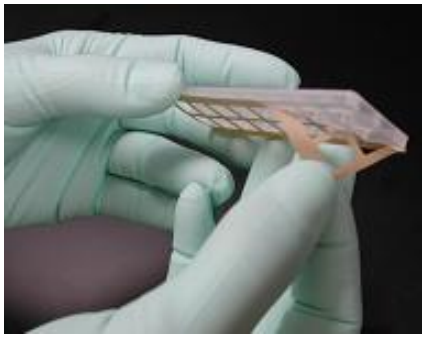
The printed protein arrays can be placed in a slide box and stored sealed at -20 °C and are at least stable for 2 months.

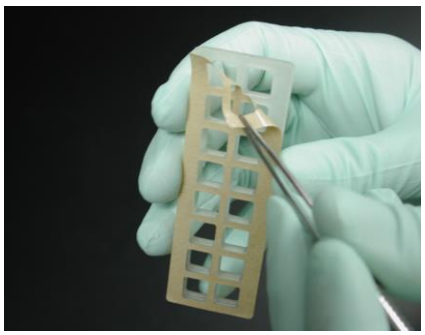
9 Washing and blocking

1. Submerge slides in Blocking Solution for 1 hour at room temperature. This deactivates the remaining reactive groups on the surface.
2. Remove slides from Blocking Solution.
3. Rinse the arrays in diH_2O.
4. Dry the arrays in an oil-free air or nitrogen stream or centrifuge (5 min at 200 x g) to avoid any water stains on the slide surface.
5. Proceed to hybridization.

10 Hybridization

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



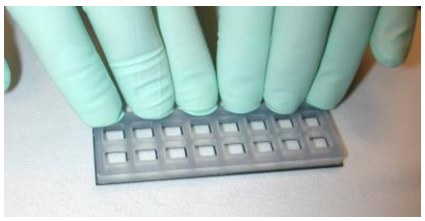


Apply the superstructure on the slide a) from top or b) by inverting the slide on the superstructure, followed by c) firmly securing the superstructure on the slide.

a) Application from top

b) Application in inverted position

c) Securing the superstructure



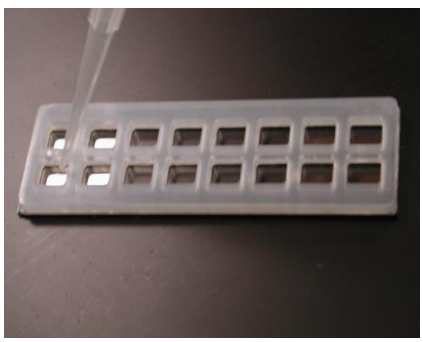
2. Resuspend the dried, labeled target in an appropriate amount of 1x hybridization solution.

   Note: At least 7.5 pmol of each target in 30 µl of hybridization buffer per well is recommended.

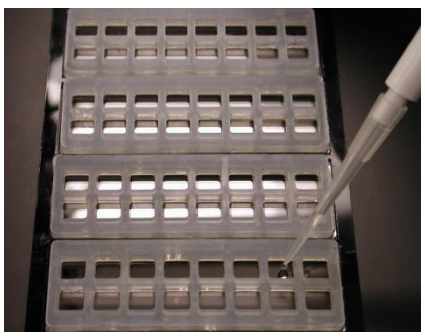
3. Denature the target by heating at 95 °C for 3 min in a water-filled well of a heat block, perform a quick spin in a micro-centrifuge, then pipette the appropriate volume into the wells of a) individual slide or b) 4 slides placed in a molded tray.

   Caution: Placing the sample on ice after hybridization may cause SDS precipitation, which may interfere with hybridization. Place the target in a 42 °C water-filled well of a heat block, if hybridization cannot be conducted immediately after denaturation. Work rapidly when adding the target; use of filter tips is recommended.

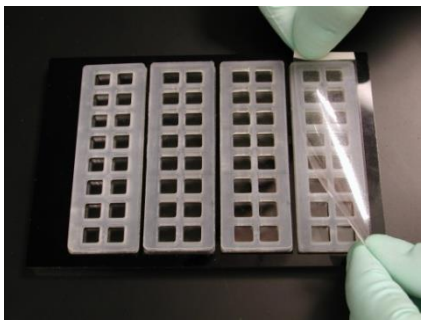
a) Addition of target to a single slide

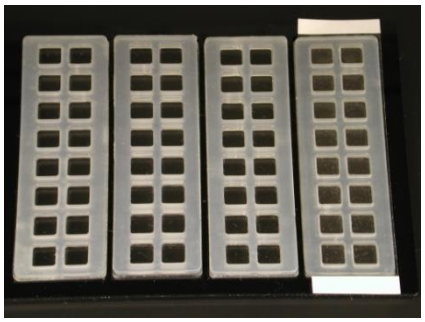


b) Addition of target to multiple slides in a tray



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





5. Hybridize the slides overnight at the appropriate temperature depending on the hybridization buffer on an orbital shaker at 100 rpm. Protect the slides by covering with Aluminium foil or other means.

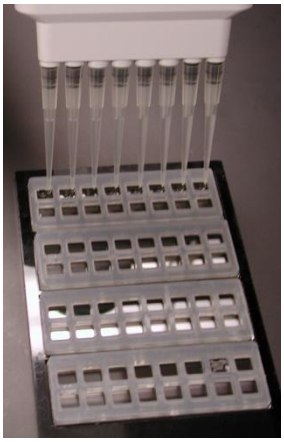
11Post-Hybridization washing

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.

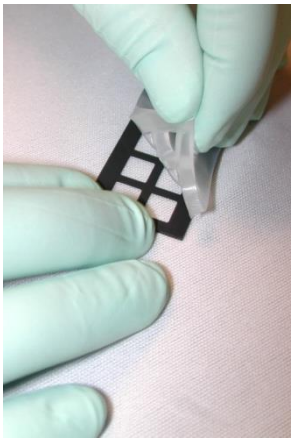
Note: The solutions recommended below for washing are a general guideline; your application may require alternative stringency washes.

1. Aspirate the target from the wells using a multi-channel pipette and immediately fill the wells with 100 µl of 2 x SSC. Aspirate the wash solution and fill the wells with fresh 2 x 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. Wash in 2 x SSC and 0.1 % SDS for 5 min, plunging gently.
4. Wash in 1 x SSC for 5 min, plunging gently.
5. Wash in 0.2 x SSC for 5 min, plunging gently.
6. Wash in 0.05 x SSC for 5 min, plunging gently.
7. Dry the arrays in an oil-free air or nitrogen stream or centrifuge (5 min at 200 x g) to avoid any water stains on the slide surface.
8. Protect the array from light, dust and handling 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.