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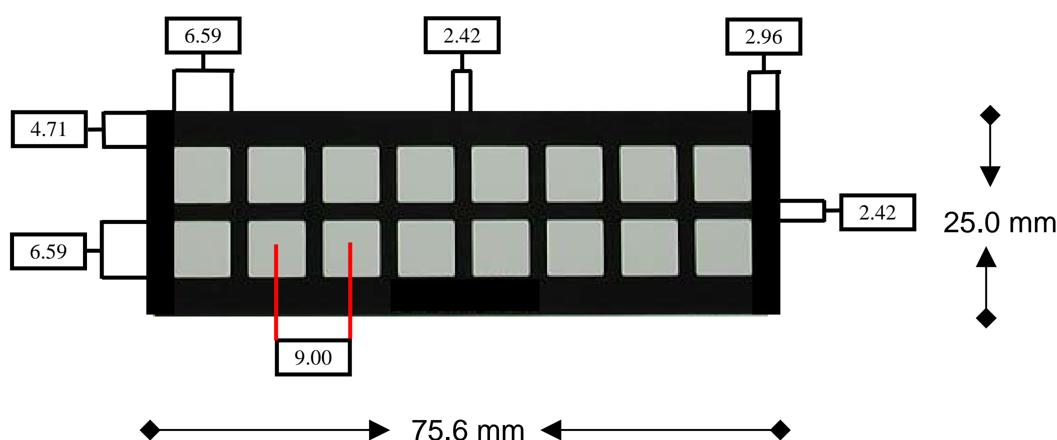
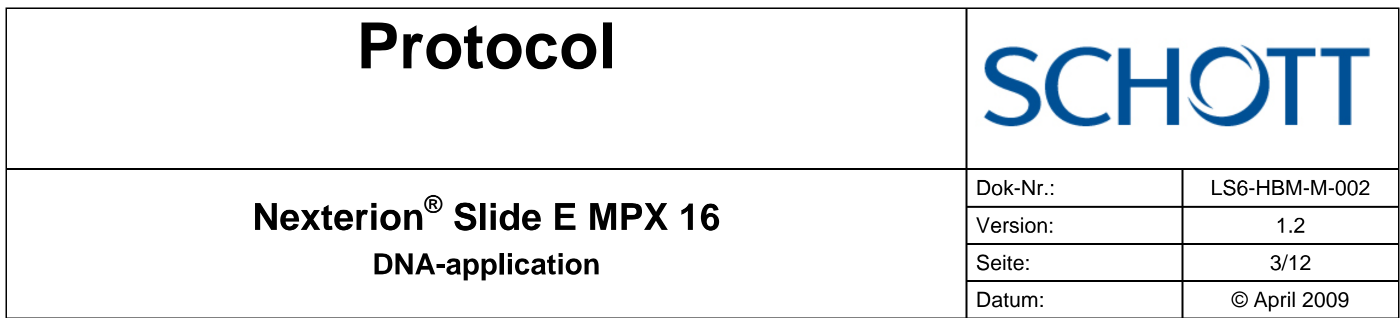
1 Introduction

Nexterion® Slide E 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 E 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.
The epoxy surface coating allows for the efficient covalent and directed binding of molecules, such as oligonucleotides and/or PCR-products. Although use of amino-modified molecules is recommended, this modification is not essential. PCR-products or oligonucleotides react with the epoxy modified glass surface to form a covalent bond. The density of epoxy groups is uniform over the entire surface of slides and is adjusted to yield optimal binding. The uniform and ultrahydrophobic patterning material separates the slide surface into 16 individually addressable subarray chambers allowing multiplexed microarray analysis. Nexterion® Slide E 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 E MPX 16 are available separately. Each MPX-4 tray can hold up to four slides.





2 Storage and handling

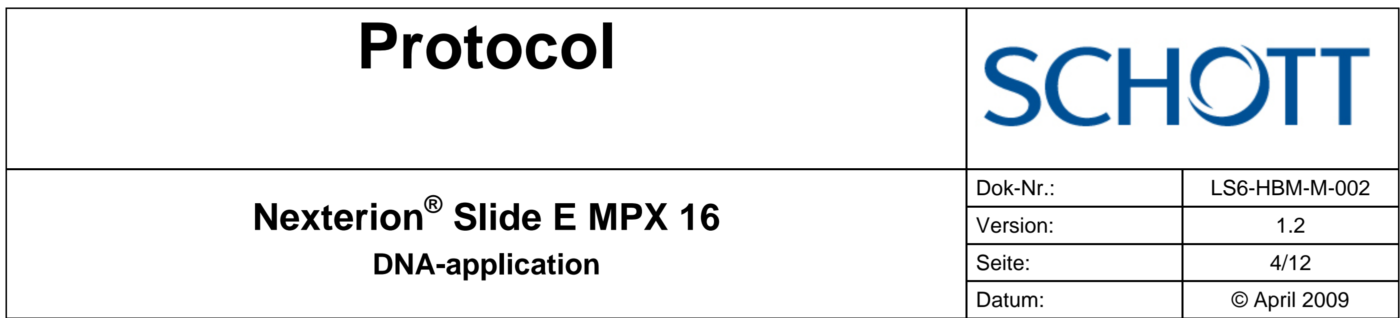
1. Store the packaged substrates at room temperature (20 - 25 ºC) and use prior to the expiration date.
2. Open and use the substrates in a clean environment to avoid particle build-up on the printing surface.
3. Avoid direct contact with the printing surface to minimize contamination and abrasion of the coated surface.
4. Once the package is opened, substrates should be used within 8 weeks if stored under inert condition inside a desiccator 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

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 E 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 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)
5. Sodium Dodecyl Sulfate (SDS)
6. Triton® X-100 solution (1ml Triton® X-100 in 1000 ml diH_2O, dissolve by heating up to 60 °C to get 0.1 % solution)
7. HCl solution (Add 100 µl 37 % HCl to 1000 ml diH_2O to get 1 mM concentration)
8. KCl solution (Dilute 100 ml 1 M KCl stock solution to 1000 ml diH_2O to obtain 100 mM concentration)
9. Nexterion® Blocking Solution (Dilute 100 ml 4 x Nexterion® Block with 300 ml diH_2O and add 80 µl of 37 % HCl to get 1 x solution)



5 Equipment required

1. Heat block capable of heating to 95 °C
2. Heated water bath
3. Compressed nitrogen gas for drying slides
4. Coplin jars (VWR 25457-006) or slide dish and rack combo (Fisher 900200) for washing slides

6 Array printing

1. Mix equal amounts of oligonucleotide probe or PCR product and 2 x Nexterion® Spot Solution 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 - 0.5 µM (approx. 0.1 - 1 mg/ml)

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, sarcosyl, Tween® 20 or SDS) may be added to Nexterion® Spot, with a final concentration between 0.01 % and 0.1 % in the 2 x 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 exo-cyclic 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 prior to spotting.
h) Amino-modified oligonucleotides are immobilized more efficiently than un-modified 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 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 the substrates at 40 - 50 % relative humidity at 20 to 25 °C.

7 Printing guidelines

Nexterion® Slide E 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 E 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.

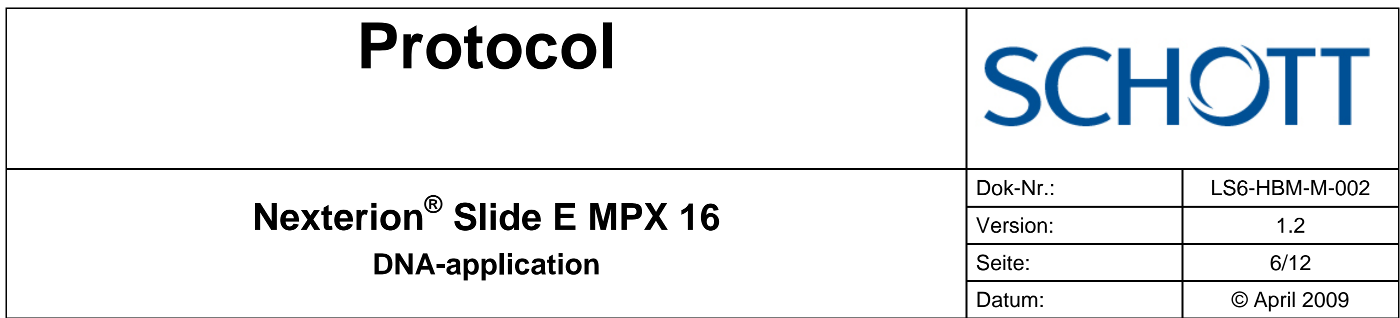
Detailed printing guidelines are available online at:
www.schott.com/nexterion

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 distance is 300 µm + pitch from well borders.



8 DNA immobilization

Note:
The first step is obligatory for all probe types and spotting solutions. The second step has to be performed in addition to the first step only for the respective probe type (as indicated) or when using betaine-containing spotting solutions (i.e. 3 x SSC containing 1.5 M betaine).

Obligatory step for all probe types and spotting solutions:

1. Incubate printed microarray slides in humidity chamber (> 90 % relative humidity) at room temperature for 30 min for completion of the covalent binding of DNA-probes on the slide surface.

Additional second step depending on probe type and spotting solution:

2. For immobilization of ***amino-modified PCR-products***, incubate the printed microarray slides at 60 °C for 30 min after the humidity chamber treatment.
   For immobilization of ***unmodified probes***, incubate the printed microarray slides at 120 °C for 30 min after the humidity chamber treatment.
   For ***betaine-containing spotting solutions***, incubate the printed microarray slides at 60 °C to 120 °C for at least 60 min after the humidity chamber treatment for efficient immobilization of oligonucleotides or PCR-products.
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.

9 Washing

1. Wash slides to remove unbound probe molecules and buffer substances to avoid interference with subsequent hybridization experiments.
   a. Rinse 1 x 5 min in 0.1 % Triton® X-100 at room temperature.
   b. Rinse 2 x 2 min in 1 mM HCl solution at room temperature.
   c. Rinse 1 x 10 min in 100 mM KCl solution at room temperature.
   d. <optional> Denaturation step for arrays spotted with PCR-probes: 1 x 3 min in boiling diH_2O.
   e. Rinse 1 x 1 min in diH_2O 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 between washing steps and between washing and blocking.

10 Blocking

1. Block the slides with Nexterion® Block E Solution or alternatively with 50 mM ethanolamine, 0.1 % SDS (add freshly before use) in 0.1 M Tris, pH 9.0 as follows:
   a. Incubate slides 1 x 15 min in 1 x Nexterion® Block E Solution at 50 °C. The volume of blocking solution should be at least 100 ml for 5 slides.
   b. Rinse 1 x 1 min in diH_2O at room temperature.
2. Dry the Nexterion® Slide E in an oil-free air or nitrogen stream to avoid any water stains on the slide surface.
3. Proceed to hybridization.

11 Hybridization

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

2. Apply the superstructure on Nexterion® Slide E MPX 16 a) from top or b) by inverting the slide on the superstructure, followed by c) firmly securing the superstructure on Nexterion® Slide E MPX 16.

a) Application from top

b) Application in inverted position

c) Securing the superstructure

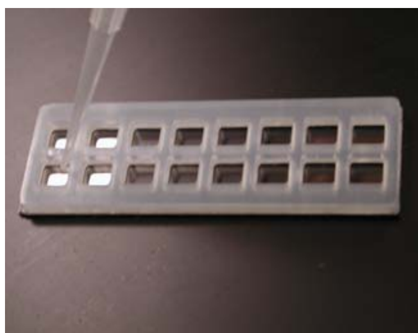
3. Re-suspend the dried, labeled target to be applied to the array in Nexterion® Hyb. In case the target is already dissolved in a different buffer or in water, the sample can also be diluted in Nexterion® Hyb to get at least 90 % (v/v) buffer in the final hybridization solution.

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

4. 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 into the wells of a) individual slide or b) 4 slides placed in a molded tray.

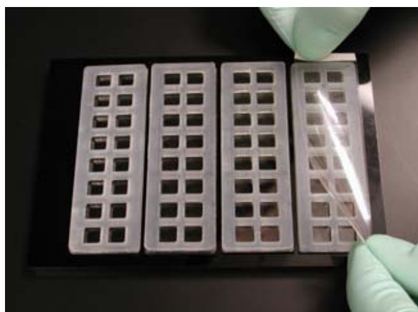
Caution:
If the sample cannot be applied immediately after denaturation, then place it in a 42 ºC water-filled well of a heat block. Work rapidly when adding the target; use of filter tips is recommended.

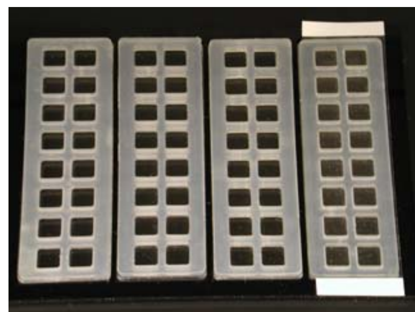
a) Addition of target to a single slide



b) Addition of target to multiple slides in a tray

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





6. Hybridize the slides overnight at 42 °C if using formamide, or 50 - 60 °C if not using formamide on an orbital shaker at 100 rpm. Protect the slides by covering with Aluminum foil or other means.

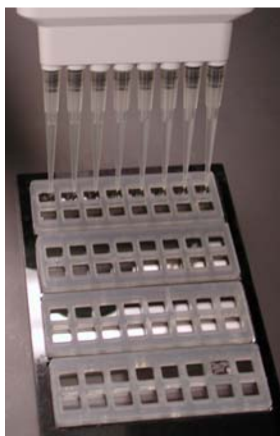
12 Post-Hybridization washing

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.

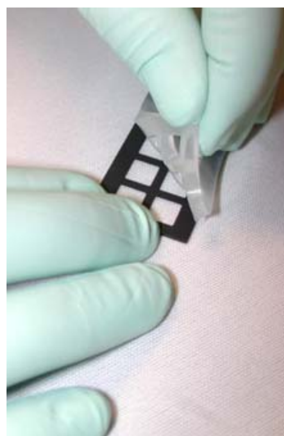
Note: The solutions recommended below for washing are a general guideline; your application may require alternative stringency washes.

1. Aspirate the target from the wells using a multi-channel pipette and immediately fill the wells with 100 µl of 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



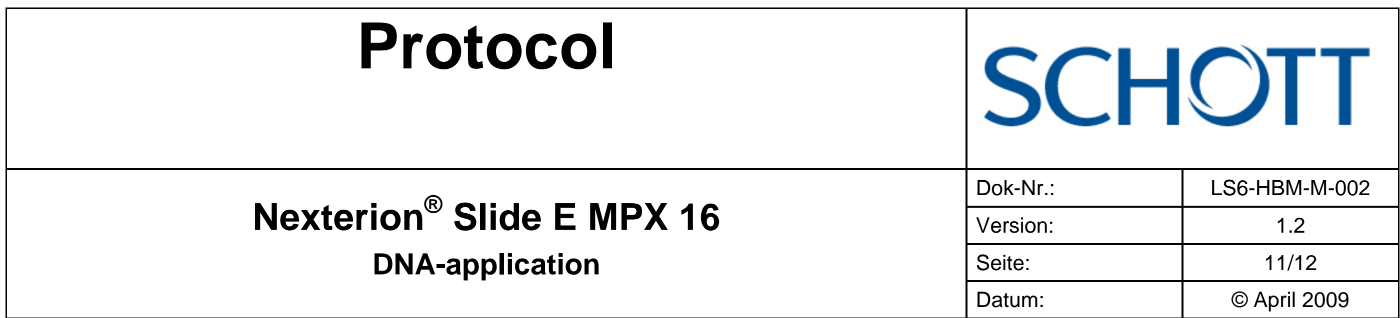
b) Removal of superstructure



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



13 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.