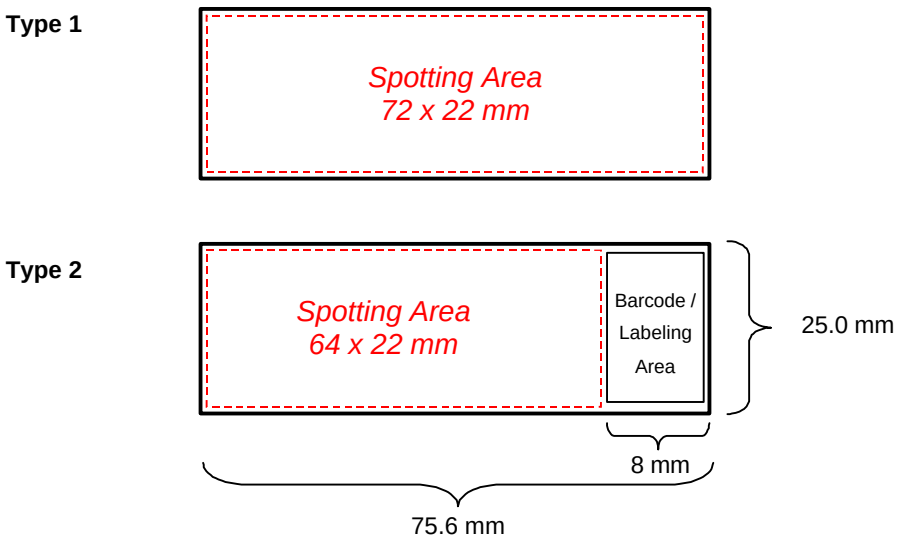
Technical Instructions for Spotting Microarrays

PRODUCT

Nexterion™ Slide AL is a special low fluorescence glass slide in the standard size of 75.6 mm x 25.0 mm x 1.0 mm. The aldehyde surface coating allows efficient covalent and directed binding of molecules with free amino-groups, e.g. synthetically fabricated oligonucleotides and/or PCR-products and/or cDNA´s and is the ideal platform for processing microarrays. For Nexterion™ Slide AL only high quality, laser-cut and extremely low-fluorescent glass substrates are used. The special cleaning and chemical coating procedures favor the generation of high-quality microarrays.
The density of aldehyde groups on the surface remains constant all over the slides and is adjusted to yield optimal binding. In combination with the spotting solution from SCHOTT Nexterion nucleic acid molecules that are dispensed with the various spotting procedures bind covalently to the glass surface.
Amino-functionalities of PCR-products or amino-modified oligonucleotides react instantly with the aldehyde modified glass surface to form a covalent bond via the Schiff´s base aldehyde-amine chemistry.
The whole surface of the Nexterion™ Slide AL is activated and can be provided also with a labeling area, e.g. a barcode to identify individual slide. The chemically reactive and homogeneous spotting area is defined for an area of 72 x 22 mm for slides without a barcode and 64 x 22 mm for slides with a barcode.



Types and Scaling of Nexterion™ Slide AL

STORAGE AND HANDLING

1. Store the packaged substrates at room temperature (20-25ºC) 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.

SCHOTT nexterion

5. The original packaging of the Nexterion™ Slide AL ideally protects the slide surface. Therefore the packaging is also good for storing the slides after the spotting and hybridization steps. After hybridization with fluorescent probes the slides additionally should be stored in dust-safe and light-protective isolating bags to avoid photo bleaching.

ARRAY PRINTING

1. Mix equal amounts of oligonucleotide probe or PCR product and 2X Nexterion Spotting 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. 0.2 – 1 mg/ml)

Notes:a) Use of the 2X Nexterion Spotting Solution is advantageous especially when spotting oligonucleotides up to a length of 50 bases.
b) Nexterion Spotting Solution I is recommended for majority of applications.
c) Nexterion Spotting Solution II is recommended for Ring-And-Pin type of microarrayers, whereas Spotting Solutions III results in slightly bigger spot diameters.
d) For Ring-And-Pin systems and for pipetting systems based on the capillary principle, a lower concentration of spotting solution from SCHOTT Nexterion could be tried.
e) Alternatively 3X SSC or 3X SSC containing 1.5 M betaine can be used as spotting buffers.
f) Do not use any spotting solution containing primary amino-groups like Tris.
g) PCR-products with amino-modified primers are preferably used for spotting. However, because of their exo-cyclic amino-groups PCR-products can be immobilized also without amino-modification.
h) When amino-functional primer is used to generate the PCR-products, the unused primers should be separated from the PCR products using a suitable method prior to spotting.
i) 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 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.

   Note: 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.

   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 coverslip and damage parts of the array.

DNA IMMOBILIZATION

1. For completion of the covalent binding of DNA-probes on the slide surface after spotting it is necessary to incubate the slides:

   a) for a period of min. 15 min in humid chamber consisting of dIH_2O at room temperature

and b) for a period of 60 - 90 min at a temperature of 120 °C.

2. 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.

WASHING AND BLOCKING

After spotting 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. Subsequently, we recommend to block the slides with Aldehyde Blocking Solution before the microarray is hybridized.

Rinse the Nexterion™ Slide AL:

1. 2 x 2 min in rinsing solution 1 at room temperature,
2. 2 x 2 min in dIH_2O at room temperature,
3. (Denaturing step for arrays spotted with PCR-probes)
   1 x 3 min in boiling dIH_2O (95 - 100°C),
4. 1 x 1 minute in dIH_2O at room temperature,
5. 1 x 15 min in Aldehyde Blocking Solution at room temperature,
6. 2 x 2 min in rinsing solution 1 at room temperature and
7. 2 x 2 min in dIH_2O at room temperature

The volume of washing solution should be at least 250 ml for 5 Nexterion™ Slide AL and the volume of Aldehyde Blocking Solution min. 100 ml for 5 Nexterion™ Slide AL.
Make sure that slides do not dry between washing steps. The final drying step of the Nexterion™ Slide AL should take place in an oil-free air or nitrogen stream or centrifuge (2 min at 150 to 200x g) to avoid any water stains on the slide surface.

HYBRIDIZATION

1. Re-suspend the dried, labeled target that will be applied to the array in Nexterion Hybridization Buffer. In case the target is already dissolved in a different buffer or in water, the sample can also be diluted in Nexterion Hybridization Buffer to get at least 90% (v/v) in the final hybridization solution (mixture ratio sample:buffer 1:9).

   Note: a) The amount of buffer depends on the desired target concentration and the size of hybridization chamber used.

   b) As an alternative to the Nexterion Hybridization Buffer, a buffer with 3–5X SSC + 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.

2. Denature the suspended target by heating at 95ºC 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 onto the array surface of a blocked slide under the cover slip or inside a hybridization chamber/station.

Caution: If the sample cannot be applied immediately after denaturation, then place it in a 42ºC water-filled well of a heat block.

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. 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.

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

4. Dry the array in an oil free air or nitrogen stream or by centrifugation at 2 min at 150 to 200x g 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. Ensure that the laser and filter set of the scanner is compatible with the fluorescent labeling of the probe molecules.

SUPPLEMENT

A. Reagents

Name	Contents	Preparation	Note
Rinsing Solution 1	0.2 % SDS	5 ml 10 % SDS + 245 ml dIH_2O	Use 10 % SDS stock solution
Aldehyde Blocking Solution	Sodium borohydride, PBS, ethanol	1.0 g $NaBH_4$ + 300 ml 1x PBS + 100 ml 99% ethanol	Use ethanol to reduce bubbling
Washing Buffer 1	2x SSC + 0.2 % SDS	25 ml 20x SSC + 5 ml 10 % SDS solution + 220 ml dIH_2O	Use 20x SSC and 10 % SDS stock solution
Washing Buffer 2	2x SSC	25 ml 20x SSC + 225 ml dIH_2O	
Washing Buffer 3	0.2x SSC	2.5 ml 20x SSC + 247.5 ml dIH_2O	
20x SSC	3 M NaCl + 0.3 M sodium citrate pH 7		Adjust pH 7 with 1 M sodium hydroxide
10 % SDS Solution	10 g dodecyl sulfate sodium salt in 100 ml dIH_2O		Dissolve at room temperature
Nexterion™Spot I, Nexterion™Spot II or Nexterion™Spot III (2x)			
Nexterion™ Hyb			

B. Online Sources

http://www.schott.com/nexterion
http://www.phrma.org
http://genomics.phrma.org
http://www.gene-chips.com
http://www.sciencemag.org
http://cmgm.stanford.edu/pbrown
http://bioinformatics.phrma.org/microarrays.html
http://www.sciborg.uwaterloo.ca/~bpbobech/Apps.html
http://sequence.aecom.yu.edu/bioinf/funcgenomic.html
http://www.nhgri.nih.gov/DIR/LCG/15K/HTML/
http://www.microarrays.org/index.html

MICROARRAY PRODUCTS

Product	Item-no.	Pack
Nexterion™ Slide AL Starter Kit 10 Nexterion™ Slide AL, 10 ml Nexterion™ Spot I, Nexterion™ Spot II and Nexterion™ Spot III, 10 ml Nexterion™ Hyb	1066026	1
Nexterion™ Slide AL, non-barcoded, 75.6 mm x 25 mm	1064874	25
Nexterion™ Slide AL, barcoded, 75.6 mm x 25 mm, with Barcode	1064876	25
Nexterion™ Spot I (2x), 10 ml	1066028	1
Nexterion™ Spot I (2x), 100 ml	1066029	1
Nexterion™ Spot II (2x), 10 ml	1066061	1
Nexterion™ Spot II (2x), 100 ml	1066062	1
Nexterion™ Spot III (2x), 10 ml	1066063	1
Nexterion™ Spot III (2x), 100 ml	1066063	1
Nexterion™ Hyb, 10 ml	1066073	1
Nexterion™ Hyb, 100 ml	1066075	1
Nexterion™ Hyb, 500 ml	1066077	1

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