Technical Instructions for Spotting Microarrays

PRODUCT OVERVIEW

Nexterion™ Slide A+ is especially suitable for efficient immobilization of PCR products, cDNA molecules and longer, synthetic oligonucleotides (size ≥ 50 mer). The product properties are optimized to support reliable microrarray hybridization results and data analysis by providing high signal intensities and excellent spot morphology.

Nexterion Slide A+ 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 allows scientists to change PCR product and/or cDNA probes to longer oligonucleotide probes without changing the surface chemistryand protocol. A re-optimization of the entire process for the oligonucleotide applications is not necessary. Thus Nexterion Slide A+ allows an economic transfer not only from PCR products and/or cDNA molecules to longer oligonucleotides, but also from any aminosilane slide to Nexterion Slide A+.

The immobilization of DNA probes to Nexterion Slide A+ 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+. A non-directed, irreversible immobilization is then achieved by UV-cross-linking. Amino-modification of the nucleic acids is not required, but such modification will not interfere with immobilization.

High signal-to-noise ratios are obtained with Nexterion Slide A+, due to the low inherent auto fluorescence, and highly efficient multipoint attachment, especially when working with PCR products, cDNAs and longer oligonucleotides. Even low intensity signals, for instance from only weakly expressed genes, can reliably be detected. To ensure a constant high and reproducible quality of Nexterion Slide A+, intensive quality control tests are performed.

The following technical instructions are valid for PCR-Products, cDNA and longer oligonucleotides.

STORAGE AND HANDLING

1. Store the packaged substrates at room temperature (20-25ºC)
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. Nexterion Slide A+ is stable for at least 3 months in the original packaging. Once the packaging is opened, substrates should be used immediately, but should be stable for up to 8 weeks if stored at room temperature, under inert conditions and protected from light.

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.

1. Refer to manufacturer supplied Material Safety and Data Sheets (MSDS) for proper handling and disposal of all chemicals.

SCHOTT nexterion

2. All Nexterion™ products are intended to be used for customer's own internal research purposes only and may not be used for drug development, drug purposes or diagnostic purposes, or for human use or human diagnostics nor may they be administered to humans in any way. Nexterion products and components thereof may not be resold, modified for resale, or used in any manner in the manufacture of commercial products without prior written approval of SCHOTT. Extreme care and exact attention should be practiced in the use of the Nexterion products.

REAGENTS REQUIRED

1. Deionized water (dIH_2O)- at least 18.2 Megohms-cm resistance is recommended
2. 2X spotting solution Nexterion™ Spot or 3X SSC, 1.5M betaine in 3X SSC or 50% DMSO
3. Hybridization buffer from SCHOTT Nexterion (formamide-free) or 3 –5X SSC + 0.1 % SDS with or without competitor DNA and formamide
4. Saline Sodium Citrate (20X SSC)- Ambion 9673
5. Sodium Dodecyl Sulfate (SDS)- Fisher BP166-500 or 10 % SDS solution for washing (10 g dodecyl sulfate sodium salt in 100 ml dIH_2O, dissolve at room temperature)
6. 0.1% SDS (10ml 10 % SDS solution in 1000 ml dIH_2O)
7. Amino Blocking Solution (5 g succinic anhydride + 315 ml n-methylpyrrolidone + 35 ml 0.2 M sodium-borate pH 8. Add sodium-borate freshly before use.)
8. Pre-Hybridization Buffer (3 –5xSSC, containing 0.1% SDS and 0.1 mg/ml BSA) or alternatively 25 ml Nexterion Hyb + 25 ml dIH_2O+ 500 mg BSA (Volume for 5 slides)

EQUIPMENT REQUIRED

1. UV crosslinker (Stratagene Stratalinker)
2. Heat block- capable of heating to 95°C
3. Heated water bath
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

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 - 20 µM**
PCR Products	**0.05 - 0.5 µM** (approx. 0.1 – 1 mg/ml)

Spotting solutions commonly used for Nexterion Slide A+:

Spotting Solution	Remark
50% DMSO	larger spot size, prevents evaporation problems during long spotting runs
3xSSC	smaller spots, standard aqueous spotting solution
3xSSC + 1.5 M betaine	larger spots, prevents evaporation problems during long spotting runs, very homogeneous spots
Nexterion Spot	smaller spots, phosphate buffer based solution

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.

4. Print substrates at 40-50% relative humidity at 20 to 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 coverslip and damage parts of the array.
   Carefully remove particles with a clean stream of compressed air or nitrogen before starting the print process.

DNA IMMOBILIZATION

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

   a) **UV-cross link** at 250 mJ
   and b) Minimum **12 h** incubation at room temperature (e.g. store slides over night)

2. Proceed to Washing

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

WASHING AND BLOCKING

After immobilization 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. To avoid bleeding of the spots it is important to perform the pre-hybridization washing steps very quickly by moving the slides (slide holder) up and down in the rinsing solution rather then using a shaker. The blocking is either done by prehybridization with BSA (protocol A, blocking past washing) or by reaction of the NH_2-groups with succinic anhydride (protocol B, blocking prior to washing).

Protocol with pre-hybridization, duration 50 min:

1. 1 x 10 to 20 sec in 0.1% SDS at room temperature
2. 1 x 10 to 20 sec 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 45 min in pre-hybridization buffer at 42°C
5. 1 x 10 to 20 sec in dIH_2O at room temperature
6. Dry slides immediately

Alternative protocol with chemical blocking, duration 20 min:

1. 1 x 15 min in Amino Blocking Solution at room temperature
2. 1 x 10 to 20 sec in 0.1% SDS at room temperature
3. 1 x 10 to 20 sec in dIH_2O at room temperature
4. (Denaturing step for arrays spotted with PCR-probes)
   1 x 3 min in boiling dIH_2O (95 - 100°C)

Dry slides immediately

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™ Hyridization 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 a buffer with 3–5X SSC + 0.1% SDS with or without competitor DNA and formamide can be used.

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.

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 6,625,225 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 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 Nexterion AG 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. To inquire about licensing under the OGT patents, please contact OGT at licensing@ogt.co.uk.

Additional information and online-ordering at:
USA/Can: www.us.schott.com/nexterion/shop
Europe/Asia: www.schott.com/nexterion/shop

For Technical Assistance please contact:

Europe / Asia – Pacific:

Schott Nexterion AG
Winzerlaer Str. 2a
07745 Jena
Germany
Phone: +49-3641-508-225
Fax: +49-3641-508-504
email: coatedsubstrate@schott.com

USA / Canada

Schott Nexterion
A Division of Schott North America Inc.
400 York Avenue
Duryea, PA 18642
USA
Phone: +1-570-457-7485, x657
Fax: +1-570-451-2059
email: coatedsubstrate@us.schott.com