Technical Instructions for Spotting Microarrays

PRODUCT OVERVIEW

Nexterion™ Slide E is manufactured using the highest quality glass, which has an ultra-flat surface, low slide-to-slide thickness variation and extremely low inherent fluorescence. The epoxy surface coating allows efficient covalent and directed binding of molecules, e.g. synthetically fabricated oligonucleotides and/or PCR-products. This includes amino modified molecules, though modification is not required. PCR-products or oligonucleotides react instantly with the epoxy modified glass surface to form a covalent bond. The special cleaning and chemical coating procedures favor the generation of high-quality microarrays. The density of epoxy groups on the surface remains constant all over the slides and is adjusted to yield optimal binding.

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.

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.
2. Nexterion Slide E is for research use only, not for in vitro diagnostic use.

REAGENTS REQUIRED

1. Deionized water (dIH_2O) - at least 18.2 Megohms-cm resistance is recommended
2. 2X spotting solutions Nexterion Spot I, Nexterion Spot II, or Nexterion Spot III or 3X SSC or 1.5 M betaine in 3X SSC
3. hybridization buffer from SCHOTT Nexterion or 3–5X SSC + 0.1 % SDS
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% Triton-X100 (1ml Triton-X100 in 1000 ml dIH_2O, dissolve by heating up to 60°C)
7. HCl solution (Add 100 µl 37% HCl to 1000 ml dIH_2O)
8. KCl solution (Dilute 100 ml 1 M KCl stock solution to 1000 ml dIH_2O)
9. 4X Nexterion Blocking Solution (Dilute 100 ml 4X blocking solution with 300 ml dIH_2O and 80 µl of 37% HCl to get 1X solution)

EQUIPMENT REQUIRED

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

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) 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.

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. Incubate printed microarray slides in humidity chamber consisting of dIH_2O for a) 30 min for binding of amine-modified oligonucleotides or b) 30 min for binding of amine-modified PCR products, followed by an exposure to 60°C for 30 min.
When using unmodified probes, incubate the printed microarray slides at 120°C for 30 min in addition to the above treatment. For betaine-containing spotting solutions (i.e. 1.5 M betaine in 3X SSC), the microarrays should be incubated at 60° to 120° C for at least 60 min for efficient immobilization of oligonucleotides or PCR-Products.

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

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-X100 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. Rinse 1 x 1 min in dIH_2O at room temperature

Notes: a) If you have spotted PCR probes, an additional denaturing step after KCl rinse is essential. Boil slides 1 x 3 min in dIH_2O at 95 - 100°C.
b) 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.

BLOCKING

1. Block the slides with Nexterion Blocking 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 1X Nexterion Blocking Solution at 50°C and
   b. Rinse 1 x 1 min in dIH_2O at room temperature.

   Note: The volume of 1X Nexterion Blocking Solution should be at least 100 ml for 5 slides.
2. Dry the Nexterion Slide E 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.
3. Proceed to hybridization.

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.
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.

SCHOTT nexterion

WARRANTY and PATENTS

SCHOTT NEXTERION'S PRODUCTS HAVE BEEN SCIENTIFICALLY DEVELOPED AND ARE SOLD FOR RESEARCH PURPOSES ONLY. SCHOTT NEXTERION'S PRODUCTS ARE NOT FOR USE IN HUMAN DIAGNOSTICS OR FOR DRUG PURPOSES, NOR ARE THEY TO BE ADMINISTERED TO HUMANS IN ANY WAY. EXTREME CARE AND EXACT ATTENTION SHOULD BE PRACTICED IN THE USE OF THE MATERIALS DESCRIBED HEREIN.
SCHOTT NEXTERION'S PRODUCTS ARE SUBJECT TO EXTENSIVE QUALITY CONTROL AND WILL PERFORM AS DESCRIBED WHEN USED PROPERLY. MANUFACTURER'S LIABILITY IS LIMITED TO THE REPLACEMENT OF THE PRODUCT, OR A FULL REFUND EXCEPT FOR WILLFUL CONDUCT OF SCHOTT NEXTERION. ANY MISUSE OF THIS PRODUCT INCLUDING THE USE OF THE WRONG PROTOCOLS IS THE FULL RESPONSIBILITY OF THE USER, AND SCHOTT NEXTERION MAKES NO WARRANTY OR GUARANTEE UNDER SUCH CIRCUMSTANCES.
ALL PRODUCTS ARE INTENDED TO BE USED FOR THE CUSTOMER'S OWN INTERNAL RESEARCH PURPOSES ONLY AND MAY NOT BE USED FOR DRUG DEVELOPMENT OR DIAGNOSTIC PURPOSES, OR FOR HUMAN USE. SCHOTT NEXTERION'S PRODUCTS AND THE 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 NEXTERION. SCHOTT NEXTERION IS IN THE PROCESS OF PATENTING CERTAIN ASPECTS OF THE TECHNOLOGY.
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 LICENCE UNDER THE FOLLOWING PATENTS: U.S. PATENT NOS. 6,040,138 OR 5,445,934 OR 5,744,305 AND UNDER THE FOLLOWING PATENTS OWNED BY OXFORD GENE TECHNOLOGY LIMITED („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'S PRODUCTS DOES NOT CONVEY ANY LICENCE UNDER ANY OF THE OGT PATENTS OR ANY OF THE OTHER PATENTS REFERRED TO. FOR THE ABOVE MENTIONED OR OTHER APPLICATIONS SCHOTT NEXTERION MAKES 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.

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