

Technical Instructions for Spotting Microarrays

PRODUCT

Nexterion™ Slide AL is an activated glass slide in the standard size 75.6 mm x 25 mm x 1mm with an aldehyde surface coating for efficient covalent and directed binding of molecules with free amino-groups, e.g. synthetically fabricated oligonucleotides and/or PCR products and/or cDNAs.

DURABILITY AND STORAGE

- Sealed 6 months at room temperature
- After unsealing store dust-free, light-protected, under dry conditions

ARRAY PRINTING

1. Mix equal amounts of oligonucleotide probe or PCR product and 2X Nexterion Spotting Solution to obtain the recommended final probe concentration (10 to 20 µM for oligonucleotides, 0.1 to 0.5 µM for PCR products).
2. Transfer an appropriate volume of probes to a microtiter plate.
3. If required (white precipitation) heat probe to 50 - 80 °C prior to spotting for 2 min
4. Setup the arrayer according to the manufacturers recommendations and print slides.

DNA IMMOBILIZATION

1. Incubated printed slides for a period of min. 15 min in a humid chamber consisting of dH₂O at room temperature
- and 2. for a period of 60 - 90 min at a temperature of 120 °C.

Intermediate storage if required (dust-free, light-protected, at room temperature and dry).

WASHING AND BLOCKING

1. 2 x 2 min in 0.2 % SDS at room temperature,
 2. 2 x 2 min in dH₂O at room temperature,
 3. (Denaturing step for arrays spotted with PCR-probes)
1 x 3 min in boiling dH₂O (95 - 100°C),
 4. 1 x 1 minute in dH₂O at room temperature,
 5. 1x 15 min in Aldehyde Blocking Solution (1.0 g NaBH₄ + 300 ml 1x PBS + 100 ml 99% ethanol, use ethanol to reduce bubbling and prepare freshly before use) at room temperature,
 6. 2 x 2 min in 0.2 % SDS at room temperature and
 7. 2 x 2 min in dH₂O at room temperature
- Quantity of washing solution: at least 250 ml for 5 Slides
 - Final drying of slides in oil-free air or nitrogen stream or centrifuge
 - The slides should never dry between wash steps (keep transfer times into next liquid < 30 sec)

The slides are now ready for following hybridization experiments.

HYBRIDIZATION

1. Direct transfer of labeled target in Nexterion Hybridization Buffer (mixing ratio sample : hybridization buffer 1:9) or as an alternative 3-5x SSC + 0.1% SDS
2. Denature the suspended target by heating at 95°C for 3 min and apply the appropriate volume onto the array surface of a blocked slide.

POST-HYBRIDIZATION WASHING

1. Wash 1 x 10 min in 2X SSC and 0.2% SDS at room temperature.
2. Wash 1 x 10 min in 2X SSC at room temperature.
3. Wash 1 x 10 min in 0.2X SSC at room temperature.
Dry the array in an oil free air or nitrogen stream or by centrifugation at 150 to 200x g for 2 min to avoid water stains on the slide surface.

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 LICENSE 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.

Complete Protocol

Please find the complete protocol at:

www.us.schott.com/nexterion

(for customers in USA)

www.schott.com/nexterion

(for customers in Europe/Asia-Pacific)

For Technical Assistance or Ordering Inquiries, please contact

Europe / Asia – Pacific:

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