Short Version

Technical Instructions for Spotting Microarrays

PRODUCT

Nexterion™ Slide E is an activated glass slide in the standard size 75.6 mm x 25 mm x 1.0 mm with an epoxy surface coating for efficient covalent and directed binding of molecules, e.g. synthetically fabricated oligonucleotides and/or PCR-products.

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. Setup the arrayer according to the manufacturers recommendations and print slides.

DNA IMMOBILIZATION

1. Incubate printed microarray slides in humidity chamber consisting of dIH2O 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 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.

WASHING

1.
   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
   e. Proceed to Blocking immediately.

   Notes: a) If you have spotted PCR probes, an additional denaturing step is essential after KCl rinse. 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 Nexterion Slide E.

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.

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.

HYBRIDIZATION

1. Re-suspend or dilute the labeled target in Nexterion Hybridization Buffer (alternatively 3-5x SSC and 0.1% SDS) to get at least 90% (v/v) in the final hybridization solution (mixture ratio sample:buffer 1:9).

2. Denature the suspended target by heating at 95ºC 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.
4. 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.

VII. 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.

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

© October 2003