Technical Instructions for Spotting DNA Microarrays

Store at –20°C prior to use. Allow package to equilibrate at room temperature before opening.

PRODUCT OVERVIEW

Nexterion™ Slide H is especially suited for the immobilization of oligonucleotides. The multi-component organic hydrogel coating provides high probe binding capacity, while the uniquely designed coating matrix inhibits non-specific binding. The three-dimensional hydrogel with amine-reactive groups allows efficient end attachment of amine-modified oligonucleotides for optimal orientation during hybridization. The combination of high-density specific attachment with a low-background matrix results in superior signal-to-noise rations in microarray experiments.

STORAGE AND HANDLING

1. The reactive groups on the Nexterion Slide H coating will undergo hydrolysis reactions if not properly protected from moisture. The slides are packaged in moisture barrier bags for shipment and storage. It is strongly recommended to store the slides at -20ºC in their original packaging prior to use, as the hydrolysis of Nexterion Slide H coating is extremely slow at low temperature. The shelf life is 6 months when stored at –20 °C.
2. The packaging should be allowed to equilibrate completely at room temperature prior to opening. Failure to do so will lead to condensation on the slide surface and loss of activity. After opening, seal any unused slides in the reusable pouch with desiccant and re-freeze.
3. Avoid direct contact with the surface of the slides to minimize contamination and abrasion of the coated surface. Always wear gloves and hold slide edge.

4. Only one side of each slide has been coated (barcoded side). The slides are nominally 25 x 75.6 x 1 mm.
5. Nexterion Slide H should be opened in a clean environment to avoid the build-up of particulate debris on the coated surface.

GENERAL PRECAUTIONS

1. The protocols contained in this document are meant to be general guidelines, and some optimization may be required depending on the specific application and sample being used.
2. Refer to manufacturer supplied Material Safety and Data Sheets (MSDS) for proper handling and disposal of all chemicals.
3. All Nexterion™ products are intended to be used for internal research purposes only and may not be used for drug development, drug purposes or diagnostic purposes, or for

4. 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. When printing at 50% relative humidity, the following buffer should be utilized :300 mM sodium phosphate (pH 8.5), 0.005% Tween20®, 0.001% sarkosyl.
2. When printing at 30% relative humidity (suggested for long print runs), the following buffer should be utilized:150 mM sodium phosphate (pH 8.5), 0.001% Tween20®
3. Print buffers should be prepared immediately before printing.
4. With most pin printers, the Tween20® concentration can be adjusted to tune the spot size; less Tween20® yields smaller spots and vice versa.
5. DMSO is *not* recommended as a co-spotting reagent
6. Reactive Group Blocking Solution: 50 mM ethanolamine in 50 mM borate buffer pH 9.0
7. 1X Hybridization Buffer: 2X SSC with 0.1% SDS and 0.1% salmon sperm DNA (formamide can be added if required)
8. Use of desalted 3' or 5' amine-modified oligonucleotides of highest purity are recommended.

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

Synthesized 3' or 5' amino-modified oligonucleotides should be desalted and purified preferably to HPLC grade to ensure that residual nucleophiles such as Tris (hydroxymethyl)-aminomethane (Tris), ethanol amine, or free ammonium ions do not inhibit coupling efficiency. *Do not dilute oligonucleotides in Tris or any other amine-containing buffer*.

1. Dilute the amine-modified oligonucleotides to a final spotting concentration of 20 μM in the printing buffer.
2. Transfer an appropriate volume of probes to a microtiter plate.
3. Setup the arrayer according to the manufacturers 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 oligonucleotides at 30% to 50% relative humidity. 30% humidity is optimal for longer print runs.
5. Post-print incubation: For best oligonucleotide coupling, place the printed arrays in a chamber maintained at 75% relative humidity for two hours, followed by storage in a vacuum desiccator for at least two hours to overnight.

Caution: The use of a diamond scriber to identify array boundaries can produce glass particulates that may become trapped under the coverslip and interfere with array performance.

WASHING AND BLOCKING

1. Submerge slides in Blocking Solution for 1 hour at room temperature. This deactivates the remaining reactive groups on the surface.
2. Remove slides from Blocking Solution.
3. Rinse the arrays in dH_2O.
4. Dry the arrays by centrifugation at 200 x g for 5 min or blow-dry.
5. Proceed to hybridization.

HYBRIDIZATION

Adjust temperature and salt concentration based on the Tm between the probe and target molecules.

1. Re-suspend the dried, labeled target in an appropriate amount of 1X hybridization solution.
2. Denature the 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.

   Caution: Placing the sample on ice after hybridization may cause SDS precipitation, which may interfere with hybridization. Place the target in a 42ºC water-filled well of a heat block, if hybridization cannot be conducted immediately after denaturation.

3. Carefully place a cover slip over the hybridization solution to cover array, avoiding the entrapment of air bubbles.

   Caution: Ensure that the cover slips are appropriate for microarray use; some cover slips may require cleaning before use.

4. Transfer to a hybridization chamber, containing sufficient dH_2O to maintain humidity, but ensure that the excess dH_2O does not come into contact with the array.
5. Place the sealed hybridization chamber into a water bath or incubator maintained at 42ºC if using formamide, or 50-60ºC if not using formamide and hybridize overnight.

   Caution: Ensure that the hybridization chamber is well sealed, as excessive drying can lead to significant background fluorescence.

POST-HYBRIDIZATION WASHING

Caution: Do not allow slides to dry between washes, and protect from light whenever possible. All washings should be performed at room temperature.

Note: The solutions recommended below for washing are a general guideline; and washes of alternative stringency may be required for some specific applications.

1. Remove the array from the hybridization chamber, taking care not to disturb the cover slip.
2. Place the array into a slide rack and immerse in a dish containing 2X SSC and 0.1% SDS. Plunge gently until the cover slip separates from the array.
3. Wash in 2X SSC and 0.1% SDS for 5 min, plunging gently.
4. Wash in 1X SSC for 5 min, plunging gently.
5. Wash in 0.2X SSC for 5 min, plunging gently.
6. Wash in 0.05X SSC for 5 min, plunging gently.
7. Dry the array by centrifugation at 200 x g for 5 min, or use compressed nitrogen gas for blow-drying.
8. Protect the array from light, dust and handling until ready for scanning.

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").
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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