Technical Instructions for Spotting Protein 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 proteins. The multi-component organic hydrogel coating provides an ideal environment for proteins and enables long-term protein stability and functionality. Amine-reactive groups in the hydrogel coating provide high probe binding capacity, while the uniquely designed coating matrix inhibits non-specific binding. 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

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. Protein Print Buffer: 300 mM sodium phosphate, pH 8.5 with 0.005% CHAPS and 100 µg/ml bovine serum albumin (see notes about protein concentration for spotting below).
2. Reactive Group Blocking Solution: 50 mM ethanolamine in 50 mM borate buffer pH 8.0.
3. Incubation Buffer and Wash Buffer I (PBST): 137mM NaCl, 2.7mM KCl, 4.3mM Na_2HPO_4, 1.4mM KH_2PO_4, pH 7.5 with 0.5% Tween20®.
4. Wash Buffer II (PBS): 137mM NaCl, 2.7mM KCl, 4.3mM Na_2HPO_4, 1.4mM KH_2PO_4, pH 7.5.

PROTEIN CONCENTRATION FOR SPOTTING

Nexterion™ Slide H provides covalent attachment of proteins though amine groups of amino acids side chains on the protein surface. The coupling efficiency of the covalent chemistry depends on a number of factors, including pH, protein print concentration, and the nature of the protein itself.

A protein probe concentration ranging from 100 to 500 µg/ml is recommended to ensure sufficient protein loading and to enable reliable and consistent assay results.

The BSA utilized in the printing buffer is to ensure good spot morphology, especially when printing lower probe concentrations. The recommended concentration of BSA works well with poly and monoclonal antibodies. However, the concentration should be reduced when working with small proteins and peptides

EQUIPMENT REQUIRED

1. Humidified hybridization chamber (like GeneMachines HybChamber) or place a 25 mm (1 inch) layer of NaCl in a chamber filled with water and cover with an airtight lid. This forms a chamber with a nominally 75% relative humidity.
2. Centrifuge with slide holders or compressed nitrogen gas for drying slides
3. Coplin jars (VWR 25457-006) or slide dish and rack combo (Fisher 900200) for washing slides

ARRAY PRINTING

Nexterion™ Slide H is compatible with all microarray printing or spotting methods, including contact printing and piezo or ink-jet technologies.

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.

Storage of printed protein arrays: Trehalose can be included in the print buffer in order to stabilize the printed protein. The printed protein arrays can be placed in a slide box and stored at 4° C.

PROTEIN IMMOBILIZATION

Print proteins at 50% relative humidity and then place arrays in a slide humidity chamber for 1 hour (this will ensure maximum coupling efficiency to surface).

WASHING AND BLOCKING

Because Nexterion™ Slide H has a reactive surface chemistry, off-feature or unspotted areas must be deactivated (blocked) before any other biomolecules are incubated with the surface. Failure to block the surface can lead to the covalent attachment of assay molecules to the Nexterion Slide H surface, thus leading to high background. The slides should be blocked after printing as described below. Due to the low nonspecific binding characteristics of the surface the use of proteins in the blocking solution is not recommended, and actually discouraged. Do not use non-fat dry milk in the blocking or assay steps.

1. Rinse the slides three times with the Wash Buffer described under section Reagents Required and one time with dH_2O. The rinses can be preformed with squeeze bottles containing the respective solutions.
   Note that lab gloves may contain residues that can contaminate the surface and can lead to increased, non-uniform background. Avoid allowing residues from the gloves to flow onto the array.
2. Submerge slides in the Reactive Group Blocking Solution (stipulated in the Reagents Required section) for 1 hour to deactivate remaining functional groups. This can be performed in a clean 50 ml conical tube or other holder designed for microscope slides. Gentle agitation can be used.
3. Remove the slides from the Blocking Solution and rinse slide three times with Wash Buffer I (stipulated in the Reagents Required section) and one more time with dH_2O. Dry the array by centrifugation at 200xg for 5 min or use compressed nitrogen gas for blow-drying.

ASSAY CONDITIONS

The printed Nexterion™ Slide H slides are robust and compatible with most conditions encountered in protein-based assays. However, an incubation buffer comprised of phosphate buffered saline with 0.5% Tween20® (also used as Wash Buffer I, see description under Reagents Required) is recommended. It is not advised to use non-fat dry milk containing buffers.
The Wash Buffer I described in the protocol above should be used between the various incubation steps in order to remove loosely bound material.

TARGET INCUBATION

1. Dilute the labeled target in an appropriate amount of incubation buffer to allow full array coverage.
2. Pipette the target containing incubation buffer onto the array surface.
3. Carefully place a cover slip over the covered 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 room temperature water bath. All incubations steps with labeled target should be carried out in the dark to avoid photo bleaching of the fluorescent dye.

WASHING

Caution: Do not allow slides to dry between washes, and protect from light whenever possible.

Note: The solutions recommended below for washing are a general guideline; alternative washes may be required depending on the application.

1. Remove the array from hybridization chamber, taking care not to disturb the cover slip.
2. Place the array into a slide rack and immerse in a dish containing Wash Buffer I. Plunge gently until the cover slip separates from the array.
3. Once the coverslips have been removed, place the arrays into a slide rack and immerse in a dish containing Wash Buffer I (PBST). Wash with shaking for 10 minutes. Repeat.
4. Wash in Wash Buffer II (PBS) for 10 minutes with agitation.
5. Dry the array by centrifugation at 200xg for 5 min or use compressed nitrogen gas for blow-drying.
6. 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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