

<h1>Protocol</h1>		
NEXTERION® Slide H Protein application	Dok-Nr.:	LS6-HBM-M-002
	Version:	2.0
	Seite:	1/6
	Datum:	© March 2013

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1 Introduction

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 manufactured using the highest quality glass (standard dimensions of 75.6 mm x 25.0 mm x 1.0 mm) and laser cutting technologies, to obtain defect and particle free slide surfaces and excellent dimensional tolerances. The slide has a very low thickness deviation, an ultra-flat surface, and an extremely low inherent fluorescence. 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 ratios in microarray experiments. Only one surface of the slide has been coated. To identify the coated side: Looking towards the slide, if it is possible to read the bar code numbers correctly you are looking at the correct surface for printing. The chemically reactive and homogeneous spotting area is defined for an area of 72 x 22 mm.

2 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 -20 °C in their original packaging prior to use, as the hydrolysis of NEXTERION[®] Slide H coating is extremely slow at low temperature. Use before expiry date.
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. NEXTERION[®] Slide H should be opened in a clean environment to avoid the build-up of particulate debris on the coated surface.

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3 General precautions

1. 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.
2. Refer to manufacturer supplied Material Safety and Data Sheets (MSDS) for proper handling and disposal of all chemicals.
3. NEXTERION® Slide H is for research use only, not for in vitro diagnostic use.

4 Reagents required

1. Protein Print Buffer: 150 mM Phosphat, pH 8.5, 0.01 % sarkosyl or Tween® 20 or NEXTERION® Spot PB (see notes about protein concentration for spotting below).
2. Blocking Solution: 100 mM phosphate buffer, 25 mM ethanol amine, 0.01 % Tween® 20, pH 8.5

Preparation:

→ stock solutions needed

100 mM Na₂HPO₄ with 25 mM ethanolamine

- dissolve 4.26 g Na₂HPO₄ in 300 ml diH₂O
 - add 0.45 ml of 100 % ethanolamine
- pH 10.7 (24 °C)

100 mM NaH₂PO₄ · 2 H₂O with 25 mM ethanolamine

- dissolve 15.6 g NaH₂PO₄ · 2 H₂O in 1000 ml diH₂O
 - add 1.50 ml of 100 % ethanolamine
- pH 5.6 (24 °C)

10 % Tween® 20 solution

- 10 ml of 100% Tween® 20 + 90 ml diH₂O

→ pH adjustment

- provide 300 ml of 100 mM Na₂HPO₄ with 25 mM ethanolamine
- add slowly about 250 ml of 100 mM NaH₂PO₄ · 2 H₂O with 25 mM ethanolamine until pH 8.5 is reached

3. Incubation Buffer and Wash Buffer I (PBST): 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, 1.4 mM KH₂PO₄, pH 7.5 with 0.05 % Tween® 20.
4. Wash Buffer II (PBS): 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, 1.4 mM KH₂PO₄, pH 7.5.

5 Equipment required

1. Cover slips (like PGC Scientific 44-596).
2. 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.

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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.

6 Protein concentration for spotting

NEXTERION[®] Slide H provides covalent attachment of proteins through amino 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 0.1 to 1 mg/ml is recommended to ensure sufficient protein loading and to enable reliable and consistent assay results.

7 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.

8 Protein immobilization

Print proteins at 50 % relative humidity and then place arrays in a slide humidity chamber (95 %) for 1 h or alternatively overnight at 75 % rel. humidity (this will ensure maximum coupling efficiency to surface).

9 Storage of printed slides

If you want to store printed arrays, please do so after printing/immobilization, but before washing/blocking. The printed protein arrays can be placed in a slide box and stored sealed at -20 °C and are at least stable for 6 months.

10 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.

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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. Submerge slides in the Blocking Solution (stipulated in the Reagents Required section) for 1 h 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.

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. Remove the slides from the Blocking Solution and rinse slides three times with Wash Buffer I (stipulated in the Reagents Required section) and one more time with diH₂O.
3. Dry the arrays in an oil-free air or nitrogen stream or by centrifugation (200 x g for 5 min) to avoid any water stains on the slide surface.

11 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 0.05 % Tween[®] 20 (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.

12 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 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 diH₂O to maintain humidity, but ensure that the excess diH₂O does not come into contact with the array.

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- 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.

13 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.

- Remove the array from hybridization chamber, taking care not to disturb the cover slip.
- 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.
- Once the cover slips 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.
- Wash in Wash Buffer II (PBS) for 10 minutes with agitation.
- Dry the array in an oil-free air or nitrogen stream or by centrifugation (200 x g for 5 min) to avoid any water stains on the slide surface.
- Protect the array from light, dust, and handling until ready for scanning.

14 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 6,225,625 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"). Other patents may apply. The purchase of SCHOTT NEXTERION® products does not convey any license under any of the OGT patents or any of the other patents referred to. For all applications SCHOTT North America Inc. and SCHOTT Technical Glass Solutions GmbH make 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. Before making or using any oligonucleotide arrays you should contact OGT to discuss a licence. To inquire about licensing under the OGT patents, please contact OGT at licensing@ogt.co.uk.