Protocol

SCHOTT

Nexterion® HiSens H **Protein application**		
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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.

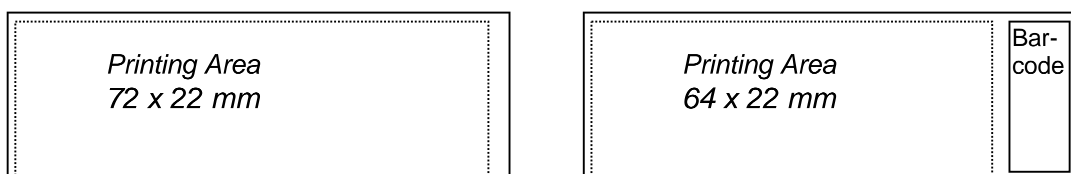
IMPORTANT NOTE

The reflective coating has only been applied to one surface of the Nexterion® HiSens slide; please consider the following remarks when using the slides:

1. Only print on the correct side of the slide (see below for instructions).
2. Do not use with scanners that image the spots through the back of the slide (for example, the Agilent scanner is not suitable for use with these slides).
3. Beware of reflected laser light if using the Nexterion® HiSens slides in an unshielded detection system.

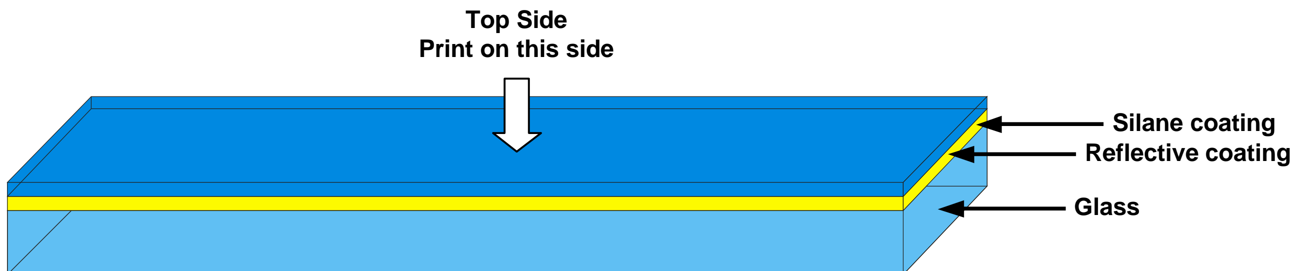
2 Product overview

Nexterion® HiSens 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 total area available for printing is 72 x 22 mm without barcode and 64 x 22 mm for slides with barcode.



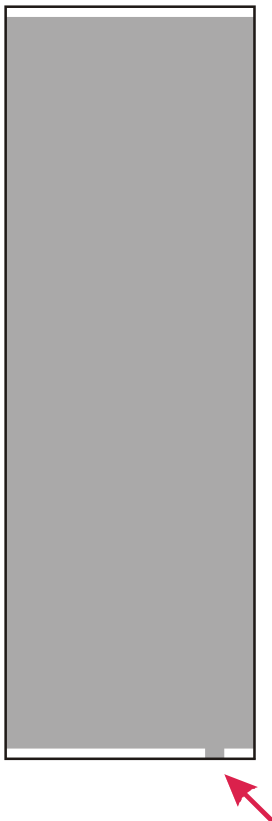
Laser cutting technologies are used to obtain a defect and particle free slide surfaces with tight dimensional tolerances. The use of stringent cleaning and sophisticated coating procedures result in the generation of high quality microarray substrates. To significantly increase signal intensities, a sophisticated reflective coating has been applied to one side of the glass. The functional chemistry coating is then applied over the reflective coating. This is a multi-component organic hydrogel coating what 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. The chemically reactive and homogeneous spotting area is defined for an area of 72 x 22 mm.

Only one surface of the Nexterion® HiSens H has been coated.

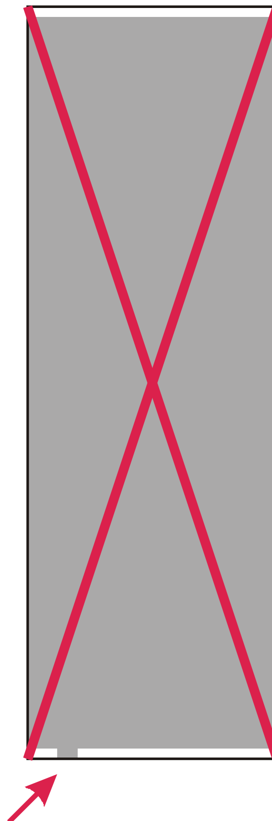


Instruction for identifying the correct side for printing
One corner of the slide is marked with a small rectangle (red arrow on figure below). When this mark is visible in the lower right corner, the side with the Nexterion® HiSens coating is on top.

correct



wrong



3 Storage and handling

1. The reactive groups on the Nexterion® HiSens 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® HiSens 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® HiSens H should be opened in a clean environment to avoid the build-up of particulate debris on the coated surface.

4 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® HiSens H is for research use only, not for in vitro diagnostic use.

5 Reagents required

1. Protein Print Buffer: 150 mM Phosphat, pH 8.5, 5 % Glycerol, 0.1 mg/ml BSA, 0.01 % Sarkosyl or Tween® 20 (see notes about protein concentration for spotting below).
2. Blocking Solution: 100 mM boric acid, 25 mM ethanol amine, 0.01 % Tween® 20, pH 8.5
   (Preparation:
   - dissolve 400 mM boric acid in 100 ml ddH$_2$O
   - dissolve 100 mM ethanol amine in 100 ml ddH$_2$O
   - dissolve 0.02 % Tween® 20 in 200 ml ddH$_2$O
   - combine all solutions, mix well, pH should be ~8.5.)
3. Incubation Buffer and Wash Buffer I (PBST): 137mM NaCl, 2.7mM KCl, 4.3mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.5 with 0.5 % Tween® 20.
4. Wash Buffer II (PBS): 137mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4,
   1.4m M KH_2PO_4, pH 7.5.

6 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.
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.

7 Protein concentration for spotting

Nexterion® HiSens H provides covalent attachment of proteins though 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 5 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.

8 Array printing

Nexterion® HiSens 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.

9 Protein immobilization

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

10 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.

11 Washing and blocking

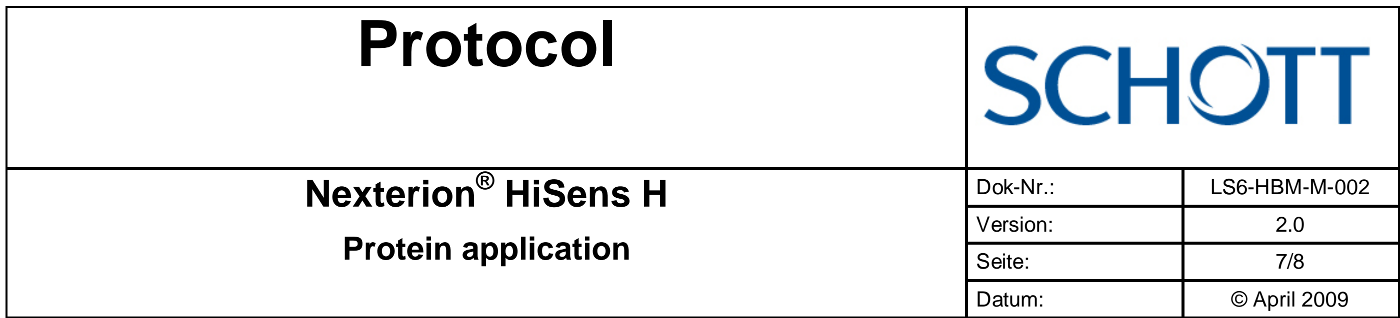
Because Nexterion® HiSens H has a reactive surface chemistry, off-feature or unspotted areas must be deactivated (blocked) before any other biomolecules are incubated with the surface.
Failure to block the surface can lead to the covalent attachment of assay molecules to the Nexterion® HiSens H surface, 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 I described under section Reagents Required and one time with diH_2O. The rinses can be performed 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 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.
3. 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 diH_2O.

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



12 Assay conditions

The printed Nexterion® HiSens 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 % 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.

13 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.

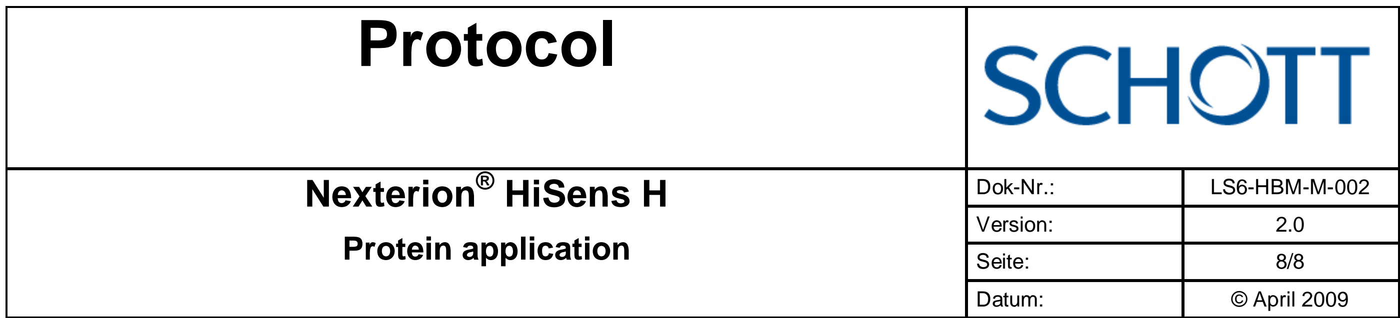
4. Transfer to a hybridization chamber, containing sufficient diH_2O to maintain humidity, but ensure that the excess diH_2O does not come into contact with the array.
5. Place the sealed hybridization chamber into a room temperature water bath. All incubations steps with labeled target should be carried out in the dark to avoid photo bleaching of the fluorescent dye.

14 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 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.



4. Wash in Wash Buffer II (PBS) for 10 minutes with agitation.
5. 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.
6. Protect the array from light, dust and abrasion of the array surface, until ready for scanning. Ensure that the scanner is compatible with the Nexterion® HiSens reflective coating and that the laser and filter set fits the fluorescent labeling of target molecules. It may be necessary to reduce the scanner detection sensitivity to avoid saturated spots.

15 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.