


<b>Protocol</b>		
<b>Nexterion® HiSens AL</b> <b>Protein application</b>	Dok-Nr.:	LS6-HBM-M-002
	Version:	2.0
	Seite:	1/6
	Datum:	© April 2009

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For Technical Assistance, please contact:

**Microarray Solutions**  
SCHOTT North America Inc.  
5530 Shepherdsville Road  
Louisville, KY 40228  
USA

Phone: +1- 502-657-4430  
Fax: +1- 502-966-4976  
E-Mail: coatedsubstrate@us.schott.com

Additional information at:  
[www.us.schott.com/nexterion](http://www.us.schott.com/nexterion)

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## 1 Introduction

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

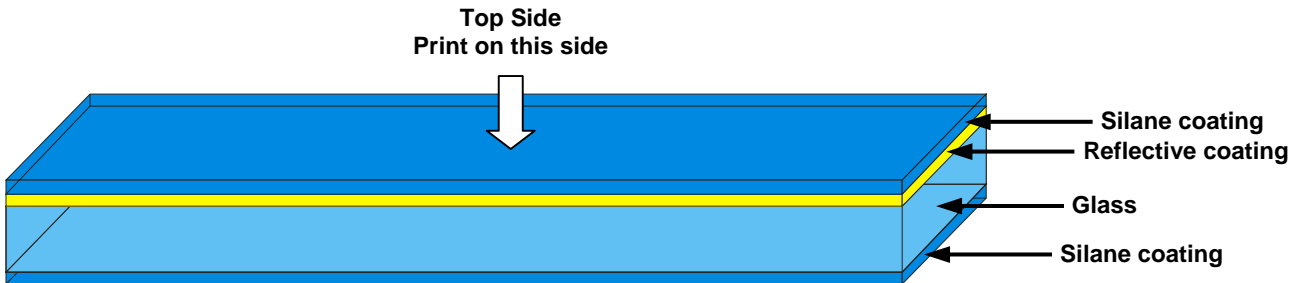
1. Only print on the correct side of the slide (see below for instructions).
2. Do not use with scanners that image the spots through the back of the slide (for example, the Agilent scanner is not suitable for use with these slides).
3. Beware of reflected laser light if using the Nexterion® HiSens slides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

## 2 Product overview

Nexterion® HiSens AL 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® HiSens AL was developed to provide the opportunity for fast and efficient coupling of proteins onto activated glass slides. The aldehyde surface coating reacts with primary amino groups provided by amino acid side chains (i.e. of lysine) immediately to form covalent bonds. Stringent cleaning and chemical coating procedures favor the generation of high-quality microarrays. The density of aldehyde groups is uniform over the entire surface of slides and is adjusted to yield optimal binding. The spotting area is defined for an area of 72 x 22 mm for slides without barcode and 64 x 22 mm for slides with barcode.



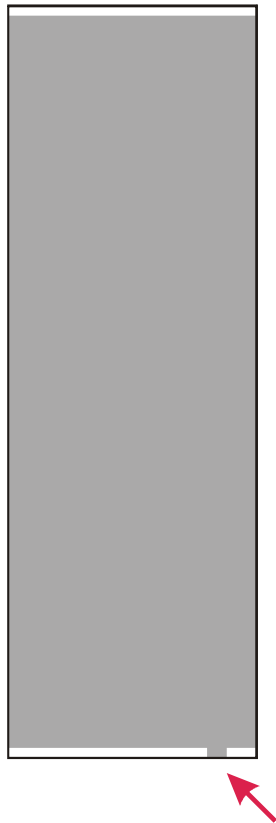
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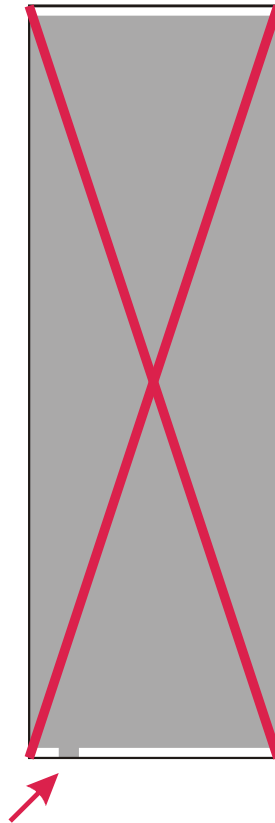
**Instructions for identifying the correct side for printing**


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



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### 3 Storage and handling

1. Store the packaged substrates at room temperature (20 - 25 °C) and use prior to the expiration date.
2. Open and use the substrates in a clean environment to avoid particle build-up on the printing surface.
3. Avoid direct contact with the printing surface to minimize contamination and abrasion of the coated surface.
4. Once the package is opened, substrates should be used within 8 weeks if stored under inert condition inside a desiccator and protected from light at room temperature.

### 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<sup>®</sup> HiSens AL is for research use only, not for in vitro diagnostic use.

### 5 Reagents required

1. Protein Print Buffer: PBS (137 mM NaCl, 9 mM KOH, 11.3 mM NaH<sub>2</sub>PO<sub>4</sub>) pH 7.0
2. Blocking Buffer: 1 % BSA in PBST (alternatively, 3 % nonfat milk in PBST can be used)
3. Wash Buffer: PBST (PBS with 0.05 % (v/v) Tween<sup>®</sup> 20 pH 7.4)
4. Rinse Solution: PBST

### 6 Equipment required

1. Humidified hybridization chamber (like GeneMachines HybChamber) or place a 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. Cover slips (like PGC Scientific 44-596).
4. Coplin jars (VWR 25457-006) or slide dish and rack combo (Fisher 900200) for washing slides.

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## 7 Array printing

Print proteins at a final concentration of 0.05 - 0.2 mg/ml in the print buffer. Nexterion® HiSens AL is compatible with all microarray printing or spotting methods, including contact printing and piezo or ink-jet technologies.

## 8 Protein immobilization

Print proteins at 50 % relative humidity and then place arrays in a humidity chamber at room temperature 1 hour.

## 9 Blocking


Block slides for one hour in blocking solution at room temperature with slow shaking.

## 10 Assay conditions

For protein incubation steps on the printed slide, we have found that PBST works well as a dilution buffer for the target solutions. The blocking buffer can also be used if an increase in nonspecific binding is observed.

## 11 Target incubation

1. Dilute the labeled target sample in an appropriate volume of incubation or blocking buffer to cover the whole array.
2. Incubate with target solution for 1 hour.
3. Remove the coverslip and place the arrays into a slide rack. Immerse in a dish containing PBST. Wash with shaking for 10 minutes. Repeat.
4. Wash in 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 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 the target molecules. It may be necessary to reduce the scanner detection sensitivity to avoid saturated spots.

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## 12 Important information about patents

Using arrays based on SCHOTT Nexterion<sup>®</sup> 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<sup>®</sup> 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](mailto:licensing@ogt.co.uk).