

# Protocol

# SCHOTT

## Nexterion® HiSens A+ DNA-application

Dok-Nr.:	LS6-HBM-M-002
Version:	2.0
Seite:	1/8
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](mailto:coatedsubstrate@us.schott.com)

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

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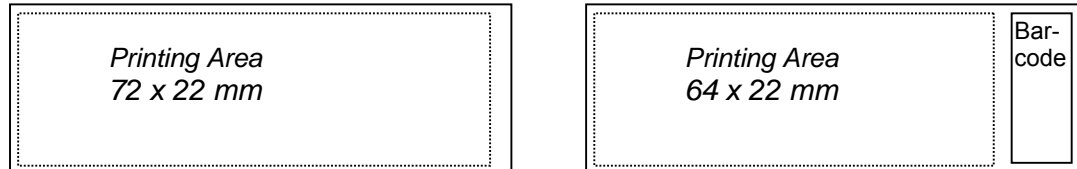
## 1 Introduction

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 A+ is manufactured from high quality glass with the standard dimensions of 75.6 mm x 25.0 mm x 1.0 mm. The total area available for printing is 72 x 22 mm without barcode and 64 x 22 mm for slides with barcode.



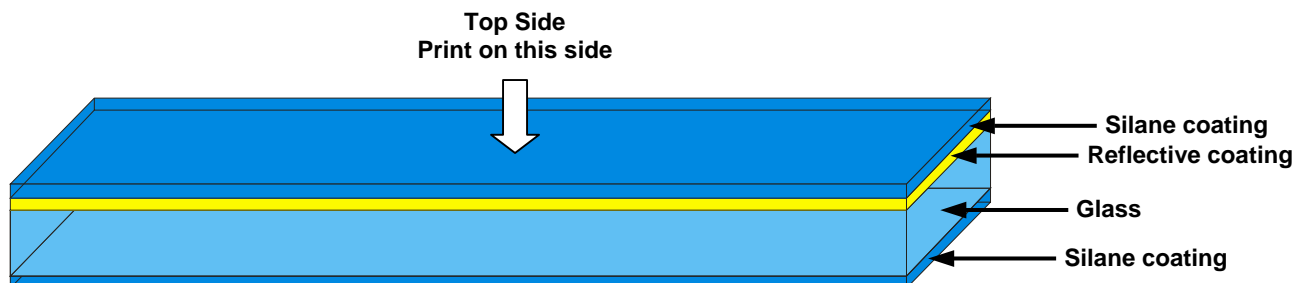
To significantly increase signal intensities a sophisticated reflective coating has been applied to one side of the glass. The functional chemistry coating is then applied over the reflective coating but due to the production method used actually covers both sides of the slide. The density of amino groups is uniform over the entire surface of slides and has been optimized to yield maximum binding. The aminosilane coating provides an ideal surface for spotting and demonstrates a high retention of unmodified longer synthetic oligonucleotides (size  $\geq$  50mer), cDNA and PCR products which are readily accessible for hybridization. The stringent cleaning and sophisticated coating procedures result in the generation of high quality microarray substrates.

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## Nexterion® HiSens A+ DNA-application

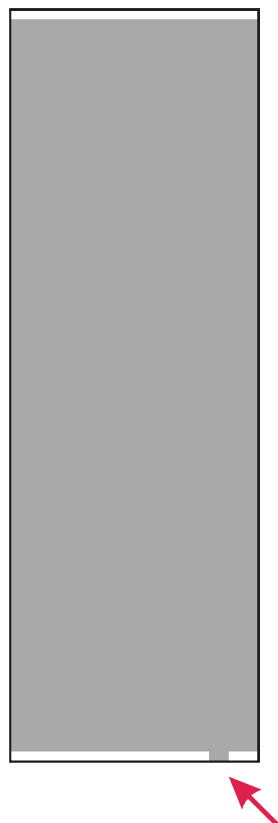
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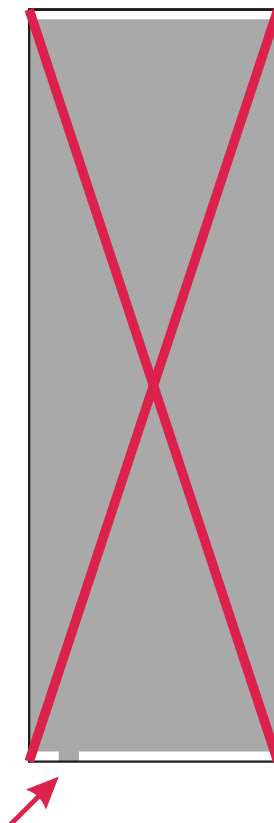
### Instructions for identifying the correct side for printing


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 chemical and reflective coatings.
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 Reagents required

1. Deionized water (diH<sub>2</sub>O).
2. 2 x Nexterion<sup>®</sup> Spotting Solution or 3 x SSC or 50 % DMSO .
3. Hybridization Buffer Nexterion<sup>®</sup> Hybridization Buffer (formamide-free) or 3 - 5 x SSC + 0.1 % SDS with or without competitor DNA and formamide.
4. Saline sodium citrate (20x SSC) - Ambion 9673 .
5. Sodium dodecyl sulfate (SDS) - Fisher BP166-500 or 10 % SDS solution for washing (10 g sodium dodecyl sulfate in 100 ml diH<sub>2</sub>O, dissolve at room temperature).
6. 0.1 % SDS (10 ml 10 % SDS solution in 1000 ml diH<sub>2</sub>O).
7. Pre-Hybridization Buffer (3 - 5 x SSC, containing 0.1 % SDS and 0.1 mg/ml BSA) or alternatively 25 ml Nexterion<sup>®</sup> Hybridization Buffer + 25 ml diH<sub>2</sub>O+ 500 mg BSA (volume for 5 slides).

### 5 Equipment required

1. UV cross-linker (Stratagene Stratalinker).
2. Heat block capable of heating to 95 °C.
3. Heated water bath.
4. Cover slips (like PGC Scientific 44-596).
5. Humidified hybridization chamber (like GeneMachines HybChamber).
6. Centrifuge with slide holders or compressed nitrogen gas for drying slides.
7. Coplin jars (VWR 25457-006) or slide dish and rack combo (Fisher 900200) for washing slides.

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## 6 Array printing

1. Dissolve oligonucleotide probe (size  $\geq 50$  mer) or PCR product in the appropriate spotting solution to obtain the recommended final probe concentration:

DNA Probes	Final Spotting Concentration
Oligonucleotides	2 - 20 $\mu$ M
PCR Products	0.1 - 1 mg/ml

Spotting solutions commonly used for Nexterion<sup>®</sup> HiSens A+:


Spotting Solution	Remark
50 % DMSO	larger spot size, prevents evaporation problems during long spotting runs
3 x SSC	smaller spots, standard aqueous spotting solution
3 x SSC containing 1.5 M betaine	larger spots, prevents evaporation problems during long spotting runs, very homogeneous spots
Nexterion <sup>®</sup> Spot	smaller spots, phosphate buffer based solution

2. Transfer an appropriate volume of probes to a microtiter plate.

**Note:** DNA-probes in Nexterion<sup>®</sup> spotting solution can be stored at -20 °C until spotting. If the probe solution shows a white precipitation prior to spotting, heat the probes to 50 to 80 °C for 2 min and avoid any change of concentration by condensation.

3. Setup the arrayer according to the manufacturer's recommendations. 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 substrates at 40 - 50 % relative humidity at 20 to 25 °C, then allow the arrays to air-dry for 10 min.

**Caution:** If you use a diamond scribe to mark the boundaries of the array, this produces small glass fragments, which may get trapped under the cover slip and damage parts of the array. Carefully remove particles with a clean stream of compressed air or nitrogen before starting the print process. Alternatively, lightly mark the boundaries of the array on the backside of the slide.

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## 7 DNA immobilization

For covalent binding of DNA-probes on the slide surface after spotting it is necessary to UV-cross link the slides at 250 mJ. Alternatively the DNA can be covalently immobilized by incubating the slides at 80 °C for 2 hours.

Incubate the slides at room temperature for 12 h (e.g. store slides over night).

Proceed to Washing


**Note:** After spotting and immobilization, the arrays can be used immediately or stored under dry, dark conditions at room temperature for several months. The washing steps after immobilization should be carried out immediately before hybridization.

## 8 Washing and Prehybridization

After immobilization it is important to remove unbound DNA-molecules and buffer substances from the slides by extensive washing to avoid any interference with subsequent hybridization experiments. To avoid bleeding of the spots it is important to perform the washing steps very quickly by moving the slides (slide holder) up and down in the rinsing solution rather than using a shaker. The blocking is done by prehybridization with BSA.

### Protocol with prehybridization, duration 50 min:

1. 1 x 10 to 20 sec in 0.1 % SDS at room temperature
2. 1 x 10 to 20 sec in diH<sub>2</sub>O at room temperature
3. (Denaturing step for arrays spotted with PCR-probes)  
1 x 3 min in boiling diH<sub>2</sub>O
4. 1 x 45 min in prehybridization buffer at 42 °C
5. 1 x 10 to 20 sec in diH<sub>2</sub>O at room temperature
6. 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.

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## 9 Hybridization

1. Re-suspend or dilute the labeled target in Nexterion® Hybridization Buffer (alternatively 3 - 5 x SSC containing 0.1 % SDS) to get at least 90 % (v/v) buffer in the final hybridization solution.

**Note:** a) The amount of buffer depends on the desired target concentration and the size of hybridization chamber used.  
b) As an alternative a buffer with 3 - 5 x SSC + 0.1 % SDS with or without competitor DNA and formamide can be used.  
c) The length of hybridization time and the hybridization temperature depend on target concentration, sequence, length, etc. and need to be optimized for each special application (i.e. 16 h 42 °C when using formamide containing hybridization buffer and 16 h 65 °C when using formamide free hybridization buffer).

2. Denature the suspended target by heating at 95 °C for 3 min in a heat block, perform a quick spin in a microcentrifuge, then pipette the appropriate volume onto the array surface of a blocked slide under the coverslip or inside a hybridization station.


**Caution:** If the sample cannot be applied immediately after denaturation, then place it in a 42 °C heat block. Work rapidly when adding the target; use of filter tips is recommended.

## 10 Post-Hybridization washing

**Caution:** Do not allow slides to dry in between washes and protect from light as much as possible. Never wash the slides with diH<sub>2</sub>O after hybridization.

**Note:** The solutions recommended below for washing are a general guideline; your application may require alternative washing stringencies. The volume of the washing solution should be at least 250 ml for 5 Slides.

1. Place the array into a slide rack and immerse in a dish containing 2 x SSC and 0.2 % SDS. Wash in the above solution 1 x 10 min at room temperature.
2. Wash 1 x 10 min in 2 x SSC.
3. Wash 1 x 10 min in 0.2 x SSC at room temperature.
4. 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.
5. 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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## 11 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](mailto:licensing@ogt.co.uk).