

Protocol

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Nexterion® HiSens H DNA-application

Dok-Nr.:	LS6-HBM-M-002
Version:	2.0
Seite:	1/8
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1 Introduction

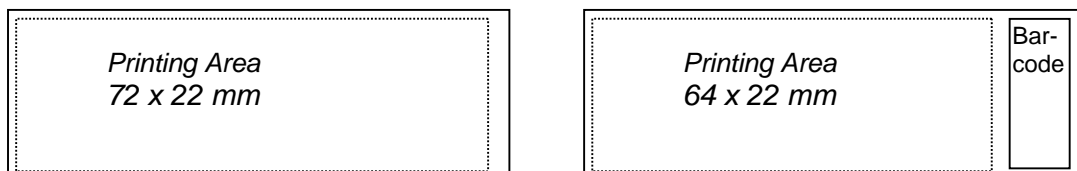
Store at -20 °C prior to use. Allow package to equilibrate at room temperature before opening.

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.



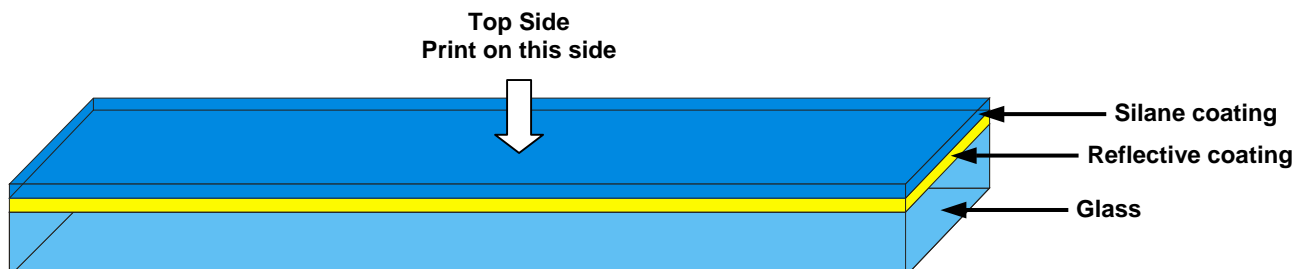
The use of stringent cleaning and sophisticated coating procedures result in the generation of high quality microarray substrates. To significantly increase signal intensities, a sophisticated reflective coating has been applied to one side of the glass. The functional chemistry coating is then applied over the reflective coating. This is a multi-component organic hydrogel coating what provides an efficient end attachment of amine-modified oligonucleotides for optimal orientation during hybridization. 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 Nexterion® HiSens H has been coated.

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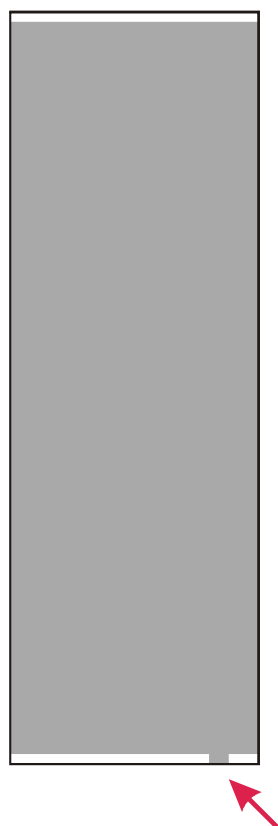
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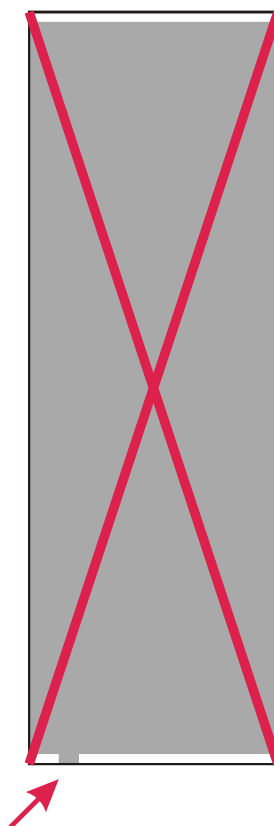
Instruction for identifying the correct side for printing


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



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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 prior to the expiration 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. When printing at 50 % relative humidity, the following buffer should be utilized: 300 mM sodium phosphate (pH 8.5) containing 0.005 % Tween® 20 and 0.001 % sarkosyl.
2. When printing at 30 % relative humidity (suggested for long print runs), the following buffer should be utilized: 150 mM sodium phosphate (pH 8.5) containing 0.001 % Tween® 20.
3. Print buffers should be prepared immediately before printing.
4. With most pin printers, the Tween® 20 concentration can be adjusted to tune the spot size; less Tween® 20 yields smaller spots and vice versa.
5. DMSO is not recommended as a co-spotting reagent

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6. 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 ddH₂O
 - dissolve 100 mM ethanol amine in 100 ml ddH₂O
 - dissolve 0.02 % Tween[®] 20 in 200 ml ddH₂O
 - combine all solutions, mix well, pH should be ~8.5.)
7. 1x Hybridization Buffer: 2 x SSC containing 0.1 % SDS and 0.1 % salmon sperm DNA (formamide can be added if required)
8. Use of desalted 3' or 5' amine-modified oligonucleotides of highest purity are recommended.

6 Equipment required

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

7 Array printing

Synthesized 3' or 5' amino-modified oligonucleotides should be desalted and purified preferably to HPLC grade to ensure that residual nucleophiles such as Tris (hydroxymethyl)-aminomethane (Tris), ethanol amine, or free ammonium ions do not inhibit coupling efficiency. **Do not dilute oligonucleotides in Tris or any other amine-containing buffer.**

1. Dilute the amino-modified oligonucleotides to a final spotting concentration of 20 μM in the printing buffer.
2. Transfer an appropriate volume of probes to a microtiter plate.
3. Setup the arrayer according to the manufacturers 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 oligonucleotides at 30 to 50 % relative humidity. 30 % humidity is optimal for longer print runs.
5. Post-print incubation: For best oligonucleotide coupling, place the printed arrays in a chamber maintained at 75 % relative humidity for two hours, followed by storage in a vacuum desiccator for at least two hours to overnight.

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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.

8 Storage of printed slides

The printed protein arrays can be placed in a slide box and stored sealed at -20 °C and are at least stable for 2 months.

9 Washing and blocking

1. Submerge slides in Blocking Solution for 1 h at room temperature. This deactivates the remaining reactive groups on the surface.
2. Remove slides from Blocking Solution.
3. Rinse the arrays in diH₂O.
4. 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.
5. Proceed to hybridization.

10 Hybridization


Adjust temperature and salt concentration based on the T_m between the probe and target molecules.

1. Re-suspend the dried, labeled target in an appropriate amount of 1x hybridization solution.
2. Denature the target by heating at 95 °C for 3 min in a water-filled well of a heat block, perform a quick spin in a micro-centrifuge, then pipette the appropriate volume onto the array surface of a blocked slide.

Caution: Placing the sample on ice after hybridization may cause SDS precipitation, which may interfere with hybridization. Place the target in a 42 °C water-filled well of a heat block, if hybridization cannot be conducted immediately after denaturation.

3. Carefully place a cover slip over the hybridization solution to cover 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.

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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.
5. Place the sealed hybridization chamber into a water bath or incubator maintained at 42 °C if using formamide, or 50 to 60 °C if not using formamide and hybridize overnight.

Caution: Ensure that the hybridization chamber is well sealed, as excessive drying can lead to significant background fluorescence.

11 Post-Hybridization washing

Note: Do not allow slides to dry between washes, and protect from light whenever possible. All washings should be performed at room temperature. The solutions recommended below for washing are a general guideline; and washes of alternative stringency may be required for some specific applications.

1. Remove the array from the hybridization chamber, taking care not to disturb the cover slip.
2. Place the array into a slide rack and immerse in a dish containing 2 x SSC and 0.1 % SDS. Plunge gently until the cover slip separates from the array.
3. Wash in 2 x SSC and 0.1 % SDS for 5 min, plunging gently.
4. Wash in 1 x SSC for 5 min, plunging gently.
5. Wash in 0.2 x SSC for 5 min, plunging gently.
6. Wash in 0.05 x SSC for 5 min, plunging gently.
7. 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.
8. 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® 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.