

Protocol

SCHOTT

Nexterion® HiSens AL DNA-application

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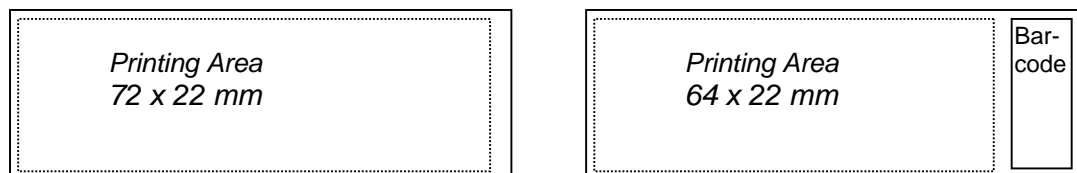
1 Introduction

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.

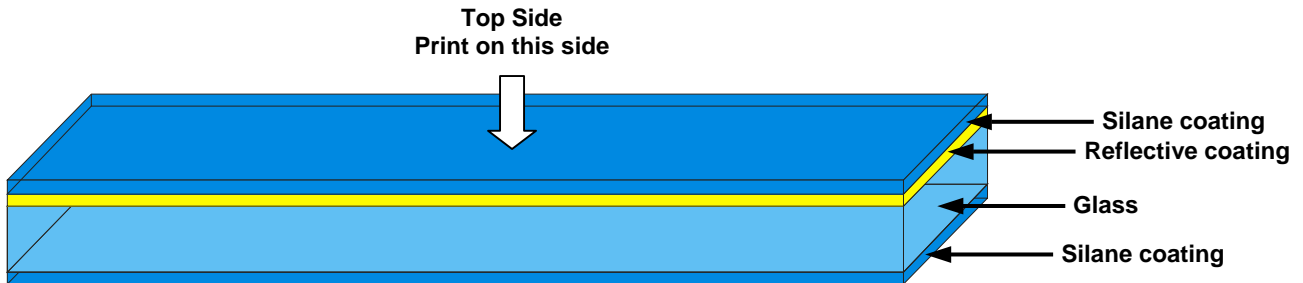
2 Product overview

Nexterion[®] HiSens AL is manufactured 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.



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, but due to the production method used, it actually covers both sides of the slide. The density of Aldehyde groups is uniform on Nexterion[®] HiSens AL and has been optimized to yield maximum binding over the entire surface of slide. Amino-functionalities of nucleic acids react with the aldehyde modified glass surface to form a covalent bond via the Schiff's base aldehyde-amine chemistry. The use of amino modified molecules is recommended, although this modification is not essential. PCR products or oligonucleotides react with the aldehyde coated surface to form a covalent bond.

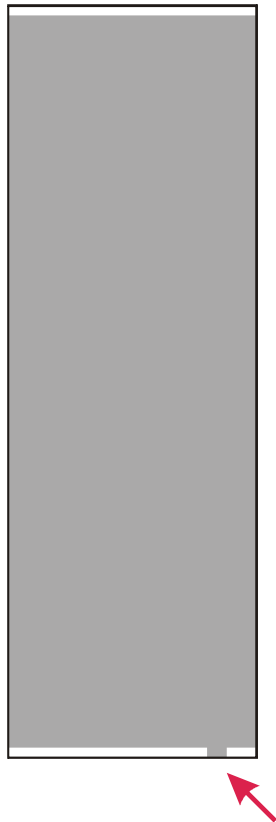
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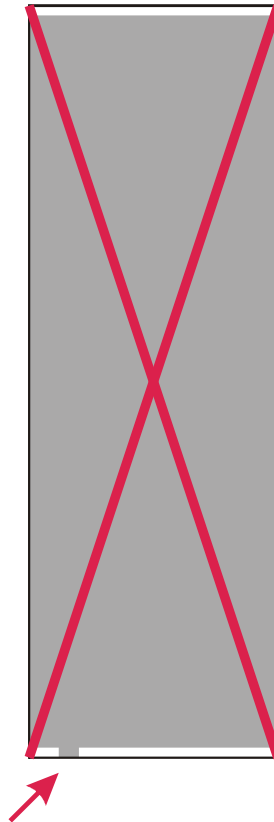
Instructions for identifying the correct side for printing


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[®] HiSens AL is for research use only, not for in vitro diagnostic use.

5 Reagents required

1. Deionized water (diH₂O) .
2. 2 x Nexterion[®] Spot Solution or 3 x SSC or 3 x SSC containing 1.5 M betaine (final concentration).
3. Hybridization Buffer Nexterion[®] Hybridization Buffer (formamide-free) or 3 - 5 x SSC containing 0.1 % SDS with or without competitor DNA and formamide.
4. Saline Sodium Citrate (20 x SSC) .
5. Sodium Dodecyl Sulfate (SDS).
6. Aldehyde Blocking Solution (Dissolve 1.0 g NaBH₄ in 300 ml PBS and 100 ml ethanol. Ethanol is used to reduce bubbling. Prepare 30 min ahead of use and do not reuse solution).

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).
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.

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7 Array printing

1. Mix equal amounts of oligonucleotide probe or PCR-product and 2 x Nexterion[®] Spot to obtain a recommended final probe concentration according to the following table:

DNA Probes	Final Spotting Concentration
Oligonucleotides	10 - 20 µM
PCR-products	0.1 – 1 µg/µl

Notes: a) Use of the Nexterion[®] Spotting Solution is recommended especially when spotting oligonucleotides.

b) For Ring-And-Pin systems and for non-contact printing systems, a lower concentration of Nexterion[®] Spot could be tried.

c) To increase the spot sizes, detergents (Cetyl-trimethylammoniumbromid, Triton[®] X-100, sarkosyl, Tween[®] 20 or SDS) may be added to Nexterion[®] Spotting Solution, with a final concentration between 0.01 and 0.1 % in the 2 x solution.

d) Alternatively, 3 x SSC or 3 x SSC containing 1.5 M betaine (final concentration) can be used as spotting buffers.

e) Do not use any spotting solution containing primary amino-groups like Tris.


f) PCR-products amplified with amino-modified primers are preferred for spotting. However unmodified PCR-products can be also immobilized, because of their exocyclic amino-groups.

g) When an amino-functional primer is used to generate the PCR-products, the unused primers should be separated from the PCR-products using a suitable method prior to spotting.

h) Amino-modified oligonucleotides are immobilized more efficiently than un-modified oligonucleotides.

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

Note: DNA-probes in Nexterion[®] Spot can be stored at -20 °C until ready for spotting. If the probe solution shows a white precipitation prior to spotting, heat the probes to 50 to 80 °C for 2 min, avoiding any change of concentration by condensation.

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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 the substrates at 40 - 50 % relative humidity at 20 to 25 °C.

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 DNA immobilization

1. Incubate printed microarray slides in humidity chamber (> 90 % relative humidity) at room temperature for 15 min for completion of the covalent binding of DNA-probes on the slide surface after spotting.
2. Incubate microarray slides at 120 °C for 60 to 90 min.
3. Proceed to washing.

Note: After spotting and immobilization, the arrays can be used immediately or stored under dry and dark conditions at room temperature. The washing steps after immobilization should not be carried out until immediately prior to hybridization.


9 Washing

1. Wash slides to remove unbound probe molecules and buffer substances to avoid interference with subsequent hybridization experiments.
 - a. Rinse 2 x 2 min in 0.2 % SDS at room temperature.
 - b. Rinse 2 x 2 min in diH₂O at room temperature.
 - c. <optional> Denaturation step for arrays spotted with PCR-probes: 1 x 3 min in boiling diH₂O.
 - d. Rinse 1 x 1 min in diH₂O at room temperature.

Note: The volume of washing solution should be at least 250 ml for 5 slides.

2. Proceed to Blocking immediately.

Note: Make sure that slides do not dry in between washing steps and between washing and blocking.

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10 Blocking

1. Block the slides with Aldehyde Blocking Solution as follows:
 - a. Incubate slides 1 x 15 min in Aldehyde Blocking Solution at room temperature. The volume of blocking solution should be at least 100 ml for 5 slides.
 - b. Rinse 2 x 2 min in 0.2 % SDS at room temperature.
 - c. Rinse 2 x 2 min in diH₂O at room temperature.
2. Dry the slides 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.
3. Proceed to hybridization.

11 Hybridization

1. Re-suspend the dried, labeled target to be applied to the array in Nexterion[®] Hyb. In case the target is already dissolved in a different buffer or in water, the sample can also be diluted in Nexterion[®] Hyb 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 coverslip used.

b) As an alternative to the Nexterion[®] Hyb, a buffer with 3 - 5 x SSC containing 0.1 % SDS 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 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 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 water-filled well of a heat block.

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12 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 diH₂O 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.

13 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.