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| <b>Protocol</b>                                      |  |               |
| <b>Nexterion® HiSens E</b><br><b>DNA application</b> | Dok-Nr.:  | LS6-HBM-M-002 |
|  | Version:  | 2.0           |
|  | Seite:  | 1/12          |
|  | Datum:  | © April 2009  |

|    |   |    |
|----|---|----|
| 1  | Introduction.....                         | 2  |
| 2  | Product overview .....                    | 2  |
| 3  | Storage and handling .....                | 4  |
| 4  | Array printing .....                      | 4  |
| 5  | DNA immobilization .....                  | 4  |
| 6  | Handling of printed arrays .....          | 5  |
| 7  | Washing .....                             | 5  |
| 8  | Blocking.....                             | 5  |
| 9  | Hybridization.....                        | 5  |
| 10 | Post-Hybridization washing .....          | 6  |
| 11 | Appendix .....                            | 7  |
| 12 | Important information about patents ..... | 12 |

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|  |               |               |
|--|---------------|---------------|
| <b>Protocol</b>                                      | <b>SCHOTT</b> |               |
| <b>Nexterion® HiSens E</b><br><b>DNA application</b> | Dok-Nr.:      | LS6-HBM-M-002 |
|  | Version:      | 2.0           |
|  | Seite:        | 2/12          |
|  | Datum:        | © April 2009  |

## 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:

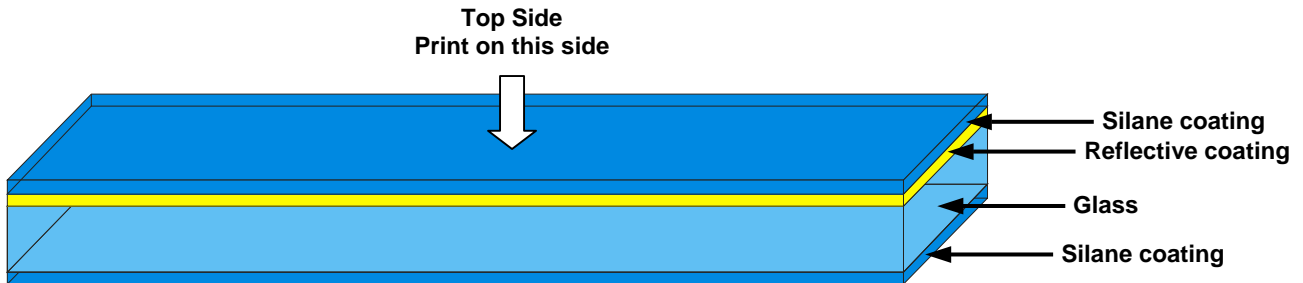
1. Only print on the correct side of the slide (see below for instructions).
2. Do not use with scanners that image the spots through the back of the slide (for example, the Agilent scanner is not suitable for use with these slides).
3. Beware of reflected laser light if using the Nexterion® HiSens slides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

## 2 Product overview

Nexterion® HiSens E 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 tight dimensional tolerances. The slide has a very low variation in thickness, an ultra-flat surface, and an extremely low inherent fluorescence. The epoxy surface coating allows for the efficient covalent and directed binding of molecules, such as oligonucleotides and/or PCR products. The use of amino modified molecules is recommended, although this modification is not essential. PCR products or oligonucleotides react with the epoxy coated glass surface to form a covalent bond. Stringent cleaning and chemical coating procedures favor the generation of high-quality microarrays. The density of epoxy 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.



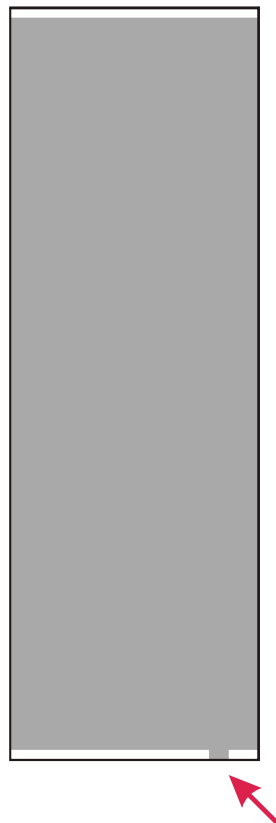
|  |               |               |
|--|---------------|---------------|
| <b>Protocol</b>                                      | <b>SCHOTT</b> |               |
| <b>Nexterion® HiSens E</b><br><b>DNA application</b> | Dok-Nr.:      | LS6-HBM-M-002 |
|  | Version:      | 2.0           |
|  | Seite:        | 3/12          |
|  | Datum:        | © April 2009  |



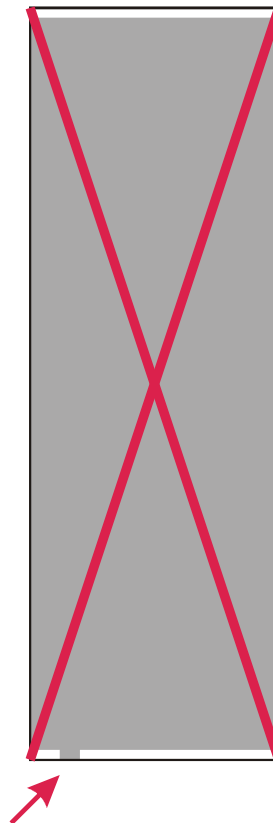
**Instructions for identifying the correct side for printing**

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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## Nexterion® HiSens E DNA application

|          |               |
|----------|---------------|
| Dok-Nr.: | LS6-HBM-M-002 |
| Version: | 2.0           |
| Seite:   | 4/12          |
| Datum:   | © April 2009  |

### 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 contamination of 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 conditions in a desiccator and protected from light at room temperature.
5. If you use a diamond scribe to mark the boundaries of the array, be aware that 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 rear of the slide.

### 4 Array printing

1. Dissolve oligonucleotide probe or PCR product in the appropriate spotting solution to obtain the recommended final probe concentration:


| DNA Probes       | Final Spotting Concentration |
|------------------|------------------------------|
| Oligonucleotides | 10 - 20 µM                   |
| PCR products     | 0.1 - 1 mg/ml                |

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

Dissolve oligonucleotide probe or PCR product in the appropriate spotting solution to obtain the recommended final probe concentration:

### 5 DNA immobilization

1. Incubate printed microarray slides in humidity chamber at room temperature for 30 min (see appendix for details of how to prepare this chamber) to ensure quantitative immobilization.
2. Proceed to washing.

|   |   |               |
|---|---|---------------|
| <b>Protocol</b>   |  |               |
| <b>Nexterion<sup>®</sup> HiSens E</b><br><b>DNA application</b> | Dok-Nr.:  | LS6-HBM-M-002 |
|   | Version:  | 2.0           |
|   | Seite:  | 5/12          |
|   | Datum:  | © April 2009  |

## 6 Handling of printed arrays

The volume of washing solution should be at least 250 ml per 5 slides. Make sure that slides do not dry out between washing steps, or between the washing and blocking step.

## 7 Washing


1. Wash slides to remove unbound probe molecules and buffer substances to avoid interference with subsequent hybridization experiments.
  - a. Rinse 1 x 5 min in 0.1 % Triton<sup>®</sup> X-100 at room temperature.
  - b. Rinse 2 x 2 min in 1 mM HCl solution at room temperature.
  - c. Rinse 1 x 10 min in 100 mM KCl solution at room temperature.
  - d. <optional> Denaturation step for arrays spotted with PCR-probes: 1 x 3 min in boiling diH<sub>2</sub>O.
  - e. Rinse 1 x 1 min in diH<sub>2</sub>O at room temperature.
2. Proceed to Blocking immediately.

## 8 Blocking

1. Block the slides with Nexterion<sup>®</sup> Blocking Solution or alternatively with 50 mM ethanolamine, 0.1 % SDS (add freshly before use) in 0.1 M Tris, pH 9.0 as follows:
  - a. Incubate slides 1 x 15 min in 1 x Nexterion<sup>®</sup> Blocking Solution at 50 °C. The volume of blocking solution should be at least 100 ml for 5 slides.
  - b. Rinse 1 x 1 min in diH<sub>2</sub>O at room temperature.
- Dry the Nexterion<sup>®</sup> Slide E 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.
- Proceed to hybridization.

## 9 Hybridization

1. Re-suspend the dried, labeled target to be applied to the array in Nexterion<sup>®</sup> 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.
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.

|  |   |               |
|--|---|---------------|
| <b>Protocol</b>                                      |  |               |
| <b>Nexterion® HiSens E</b><br><b>DNA application</b> | Dok-Nr.:  | LS6-HBM-M-002 |
|  | Version:  | 2.0           |
|  | Seite:  | 6/12          |
|  | Datum:  | © April 2009  |

## 10 Post-Hybridization washing

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.

# Protocol



## Nexterion® HiSens E DNA application

|          |               |
|----------|---------------|
| Dok-Nr.: | LS6-HBM-M-002 |
| Version: | 2.0           |
| Seite:   | 7/12          |
| Datum:   | © April 2009  |

## 11 Appendix

### 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® Slide E is for research use only, not for in vitro diagnostic use.

### Reagents Required:

| Solution/Buffer                    | Content  | Preparation  | Remark   |
|------------------------------------|--|--|--|
| Post spotting washing solutions    | 0.1 % Triton® X-100                                | 1 ml Triton® X-100, add diH <sub>2</sub> O to 1000 ml            | Warm to 60 °C to dissolve Triton® X-100, cool down before use  |
|                                    | 1 mM HCl   | 100 µl 37 % HCl, add diH <sub>2</sub> O to 1000 ml               |  |
|                                    | 100 mM KCl   | 100 ml 1 M KCl stock solution, add diH <sub>2</sub> O to 1000 ml | 1 M KCl: dissolve 74.56 g KCl in 1000 ml diH <sub>2</sub> O  |
| Blocking Solution                  | Block E, diH <sub>2</sub> O, HCl                   | Prepare as indicated on the Block E bottle                       | After addition of diH <sub>2</sub> O and HCl the solution is stable for several weeks at room temperature  |
| Hybridization Buffer               | Main ingredients: phosphate buffer, SSC, SDS, EDTA | Ready-to-use   | Does not contain formamide or competitor DNA, alternatively use 3 - 5 x SSC containing 0.1 % SDS   |
| Post hybridization washing buffers | 2 x SSC 0.2 % SDS                                  | 25 ml 20 x SSC, 5 ml 10 % SDS, add diH <sub>2</sub> O to 250 ml  | 20 x SSC: 3 M NaCl, 0.3 M sodium citrate buffer pH 7.0 (dissolve 175.3 g NaCl and 88.2 g sodium citrate in 800 ml diH <sub>2</sub> O, adjust pH to 7.0 with 10 N NaOH, add diH <sub>2</sub> O to 1000 ml |
|                                    | 2 x SSC  | 25 ml 20 x SSC, add diH <sub>2</sub> O to 250 ml                 |  |
|                                    | 0.2 x SSC  | 2.5 ml 20 x SSC, add diH <sub>2</sub> O to 250 ml                | 10 % SDS: dissolve 10 g sodium dodecyl sulphate in 100 ml diH <sub>2</sub> O   |

# Protocol



## Nexterion<sup>®</sup> HiSens E DNA application

|          |               |
|----------|---------------|
| Dok-Nr.: | LS6-HBM-M-002 |
| Version: | 2.0           |
| Seite:   | 8/12          |
| Datum:   | © April 2009  |

### Equipment Required:

1. Heated water bath.
2. Cover slips, Geneframes or Hybriwells
3. Humidified hybridization chamber (like GeneMachines HybChamber).
4. Centrifuge with slide holders or compressed nitrogen gas for drying slides.
5. Coplin jars (VWR 25457-006) or slide dish and rack combo (Fisher 900200) for washing slides.


### Spotting Solutions:

Use of the Nexterion<sup>®</sup> Spot is recommended especially when spotting oligonucleotides. For Ring-And-Pin systems and for non-contact printing systems, a lower concentration of Nexterion<sup>®</sup> Spot could be used.

Do not use any spotting solution containing primary amino-groups like Tris as these compete with the spotting material for attachment sites.

| Slide E Spotting Buffer Compatibility    |  |           |   |
|--|--|-----------|---|
| First Choice                             | Phosphate Buffer Based   | SSC Based | Low Evaporation                               |
| Nexterion <sup>®</sup> Spot <sup>1</sup> | 50 to 300 mM sodium phosphate buffer pH 8.0 to 9.0 <sup>1</sup>            | 3 x SSC   | Nexterion <sup>®</sup> Spot LE (first choice) |
|  | 300 mM Phosphate Buffer + 0.005 % Tween <sup>®</sup> 20 + 0.001 % sarkosyl |           | 3 x SSC + 1.5 M betaine                       |
|  | 150 mM Phosphate Buffer + 0.001 % Tween <sup>®</sup> 20                    |           |   |

<sup>1</sup> Add detergents to increase spot size: SDS, sarkosyl, Tween<sup>®</sup> 20, Triton<sup>®</sup> X-100, Cetyl-trimethyl-ammoniumbromide, final concentrations needs to be determined empirically, usual concentration range is between 0.001 % and 0.05 % (final concentration).

|  |   |               |
|--|---|---------------|
| <b>Protocol</b>                                      |  |               |
| <b>Nexterion® HiSens E</b><br><b>DNA application</b> | Dok-Nr.:  | LS6-HBM-M-002 |
|  | Version:  | 2.0           |
|  | Seite:  | 9/12          |
|  | Datum:  | © April 2009  |

**Can I print on both sides of the slides?**

No, as the optical Nexterion® HiSens coating is applied only on one side of the slide it is not possible to print on the back side. To determine the correct side please see “Instructions for identifying the correct side for printing” in the “Product overview” section.

**Spotting Probes:**

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

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.

Amino-modified short (up to 25 bases) oligonucleotides are immobilized more efficiently than un-modified short oligonucleotides. For longer oligonucleotides the amino-modification does not significantly improve binding efficiency.


**Immobilization:**

Baking has to be performed in addition to the incubation of printed microarrays at high rel. humidity only for the respective probe type (as indicated) or when using betaine-containing spotting solutions (i.e. 3 x SSC containing 1.5 M betaine).

| <b>Unmodified oligonucleotides and unmodified PCR products</b> | <b>All probe types dissolved in a betaine containing spotting solution</b> |
|--|--|
| Incubate the printed microarray slides at 80 °C for 120 min    | Incubate the printed microarray slides at 60 to 120 °C for at least 60 min |

**Handling of Spotting Probes:**

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 °C to 80 °C for 2 min, avoiding any change of concentration by condensation.

|  |   |               |
|--|---|---------------|
| <b>Protocol</b>                                      |  |               |
| <b>Nexterion® HiSens E</b><br><b>DNA application</b> | Dok-Nr.:  | LS6-HBM-M-002 |
|  | Version:  | 2.0           |
|  | Seite:  | 10/12         |
|  | Datum:  | © April 2009  |

### Storage of spotted slides:

There are two recommended ways to store the printed slides:

1. Store the slides directly after spotting and any immobilization steps (i.e. humidity chamber treatment and baking, if required).
2. Alternatively store the slides after the washing and blocking steps. Ensure that the slides are stored dry and in the dark, ideally sealed under an inert atmosphere or in a desiccator. For the long-term storage (a period over several months) it is better to store slides immediately after spotting, without any post printing treatment. For short-term storage (i.e. several days) the slides can be stored with or without carrying out the washing and blocking steps.

Blocked slides should not be stored in blue SCHOTT 25-slide mailers for any extended periods, as this can increase the level of background fluorescence observed after hybridization. If slides need to be stored blocked, use blue 5-slide mailer boxes instead.

### Preparation of a humidity chamber:

Prepare a suitable enclosed container (such as an acryl-glass container, plastic food container, or dessicator) by adding a tray to hold the slides and underneath this, a large Petri dish. Fill the Petri dish with steaming hot water 30 min prior to use. After 30 min the air will be saturated with water and have cooled to room temperature. The humid chamber is now ready for use. A new chamber must be prepared for each batch of slides and in advance.

An alternative option is to place a Petri dish containing saturated NaCl solution (with lots of undissolved NaCl) in a suitable sealable container. After achieving equilibration (which will take several hours), the relative humidity will be 75 % at 20 °C. This method works well and has the advantage of only needed to be prepared once. Slides may be incubated from 2 hours to over night.

### Tips for Hybridization:


The amount of buffer depends on the desired target concentration and the size of the hybridization coverslip used.

As an alternative to the Nexterion® Hyb, a buffer with 3 - 5 x SSC containing 0.1 % SDS with and without formamide can be used.

The length of the hybridization time and temperature depends on the target concentration, sequence, length, etc. and needs to be optimized for each special application (i.e. commonly used conditions are 16 h 42 °C when using formamide containing hybridization buffer and 16 h 65 °C when using formamide free hybridization buffer).

If the sample cannot be applied immediately after denaturation, then place it in a 42 °C water-filled well of a heat block.


Work rapidly when adding the target; and the use of filter pipette tips is recommended.

|   |   |               |
|---|---|---------------|
| <b>Protocol</b>   |  |               |
| <b>Nexterion<sup>®</sup> HiSens E</b><br><b>DNA application</b> | Dok-Nr.:  | LS6-HBM-M-002 |
|   | Version:  | 2.0           |
|   | Seite:  | 11/12         |
|   | Datum:  | © April 2009  |

**Tips for post-hybridization handling:**

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.

The solutions recommended above 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 per 5 Slides.

|  |   |               |
|--|---|---------------|
| <b>Protocol</b>                                      |  |               |
| <b>Nexterion® HiSens E</b><br><b>DNA application</b> | Dok-Nr.:  | LS6-HBM-M-002 |
|  | Version:  | 2.0           |
|  | Seite:  | 12/12         |
|  | Datum:  | © April 2009  |

## 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](mailto:licensing@ogt.co.uk).