SCHOTT NEXTERION® Slide H

Protocol

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For technical assistance, please contact:

SCHOTT Technical Glass Solutions GmbH

Otto-Schott-Straße 13

07745 Jena

Germany

www.schott.com/nexterion

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1. DNA application

1.1 Introduction

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

NEXTERION® Slide H is manufactured using the highest quality glass (standard dimensions of 75.6 mm x 25 mm x 1 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® Slide H is especially suited for the immobilization of amine-modified oligonucleotides. The multi-component organic hydrogel coating provides high probe binding capacity, while the uniquely designed coating matrix inhibits non-specific binding. The three-dimensional hydrogel with amine-reactive groups allows efficient end attachment of amine-modified oligonucleotides for optimal orientation during hybridization. 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 slide has been coated. To identify the coated side: Looking towards the slide, if it is possible to read the bar code numbers correctly you are looking at the correct surface for printing. The chemically reactive and homogeneous spotting area is defined for a centered area of 64 mm x 22 mm.

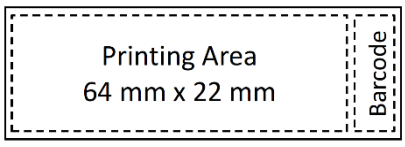

Fig 1: Printing Area

1.2 Storage and handling

1. The reactive groups on the NEXTERION® Slide H coating will undergo hydrolysis reactions if not properly protected from moisture. The slides are packaged in moisture barrier pouches for shipment and storage. It is strongly recommended to store the slides at -20 °C in their original packaging immediately upon receipt, as the hydrolysis of NEXTERION® Slide H coating is extremely slow at low temperature. Use prior to the expiration date.
2. The packaging should be allowed to equilibrate completely to room temperature prior to opening. Failure to do so will lead to condensation on the slide surface and loss of activity. Process the temperature equilibrated slides within 24 hours (spotting and immobilization). After opening, seal any unused slides in the original box in a pouch with desiccant and re-freeze immediately.
3. Avoid direct contact with the surface of the slides to minimize contamination and abrasion of the coated surface. Always wear gloves and touch only on the slide edges.
4. NEXTERION® Slide H should be opened in a clean environment to avoid the build-up of particulate debris on the coated surface.

1.3 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 needed.
3. NEXTERION® Slide H is for research use only, not for in vitro diagnostic use.

1.4 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
6. Blocking Solution: 100 mM phosphate buffer, 25 mM ethanol amine, 0.01 % Tween® 20, pH 8.5
   Preparation:
   ➔ stock solutions needed

   100 mM Na_2HPO_4 with 25 mM ethanolamine
   - dissolve 4.26 g Na_2HPO_4 in 300 ml diH_2O
   - add 0.45 ml of 100 % ethanolamine
   → pH 10.7 (24° C)

   100 nM $NaH_2PO_4 \cdot 2\ H_2O$ with 25 mM ethanolamine
   - dissolve 15.6 g $NaH_2PO_4 \cdot 2\ H_2O$ in 1000 ml diH_2O
   - add 1.5 ml of 100 % ethanolamine
   → pH 5.6 (24° C)

   10% Tween® 20 solution
   - 10 ml of 100% Tween® 20 + 90 ml diH_2O

   ➔ pH adjustment

   - provide 300 ml of 100 mM Na_2HPO_4 with 25 mM ethanolamine
   - add slowly about 250 ml of 100 mM $NaH_2PO4 \cdot 2\ H_2O$ with 25 mM ethanolamine until pH 8.5 is reached
   - finally add appropriate volume of 10 % Tween® 20 (0.55 ml for 550 ml solution)
7. 1 x 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 is recommended.

1.5 Equipment required

1. Heat block capable of heating to 95° C.
2. Heated water bath.
3. Cover slips (like Menzel BBAD02400600#AC or Marienfeld 0102242).
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), Shandon™ Horizontal Staining Jars (Thermo Scientific #133) or similar for washing slides.

1.6 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 (Tris (hydroxymethyl)-aminomethane)), 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 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 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 at least two hours to overnight.

Caution: If you use a diamond scriber 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.

1.7 Storage of printed slides

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

1.8 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 diH_2O.
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.

1.9 Hybridization

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

1. Re-suspend the dried, labeled target in an appropriate amount of 1 x 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.
4. Transfer to a hybridization chamber, containing sufficient diH_2O to maintain humidity, but ensure that the excess diH_2O does not come into contact with the array.
   Place the sealed hybridization chamber into a water bath or incubator maintained at 42 °C if using formamide, or 50 to 60 °C (dependent on the annealing temperature) 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.

1.10 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 containing 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 handling until ready for scanning.

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2. Protein application

2.1 Introduction

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

NEXTERION® Slide H is manufactured using the highest quality glass (standard dimensions of 75.6 mm x 25 mm x 1 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® Slide H is especially suited for the immobilization of proteins. The multi-component organic hydrogel coating provides an ideal environment for proteins and enables long-term protein stability and functionality. 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 slide has been coated. To identify the coated side: Looking towards the slide, if it is possible to read the bar code numbers correctly you are looking at the correct surface for printing. The chemically reactive and homogeneous spotting area is defined for an area of 64 mm x 22 mm.

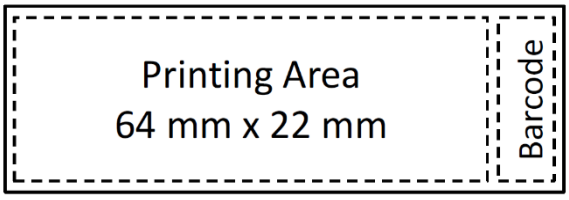

Fig 1: Printing Area

2.2 Storage and handling

1. The reactive groups on the NEXTERION® Slide H coating will undergo hydrolysis reactions if not properly protected from moisture. The slides are packaged in moisture barrier pouches for shipment and storage. It is strongly recommended to store the slides at -20 °C in their original packaging immediately upon receipt, as the hydrolysis of NEXTERION® Slide H coating is extremely slow at low temperature. Use prior to the expiration date.
2. The packaging should be allowed to equilibrate completely to room temperature prior to opening. Failure to do so will lead to condensation on the slide surface and loss of activity. Process the temperature equilibrated slides within 24 hours (spotting and immobilization). After opening, seal any unused slides in the original box in a pouch with desiccant and re-freeze immediately.
3. Avoid direct contact with the surface of the slides to minimize contamination and abrasion of the coated surface. Always wear gloves and touch only on the slide edges.
4. NEXTERION® Slide H should be opened in a clean environment to avoid the build-up of particulate debris on the coated surface.

2.3 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 needed.
3. NEXTERION® Slide H is for research use only, not for in vitro diagnostic use.

2.4 Reagent required

1. Protein Print Buffer: 150 mM phosphate, pH 8.5, 0.01 % sarkosyl or Tween® 20 or NEXTERION® Spot PB (see notes about protein concentration for spotting below).
2. Blocking Solution: 100 mM phosphate buffer, 25 mM ethanol amine, 0.01 % Tween® 20, pH 8.5
   Preparation:
   ➔ stock solutions needed
   100 mM Na_2HPO_4 with 25 mM ethanolamine
   - dissolve 4.26 g Na_2HPO_4 in 300 ml diH_2O
   - add 0.45 ml of 100 % ethanolamine

   → pH 10.7 (24° C)

   100 mM $NaH_2PO_4 \cdot 2\ H_2O$ with 25 mM ethanolamine
   - dissolve 15.6 g $NaH_2PO_4 \cdot diH_2O$ in 1000 ml diH_2O
   - add 1.5 ml of 100 % ethanolamine

   → pH 5.6 (24° C)

   10 % Tween® 20 solution
   - 10 ml of 100% Tween® 20 + 90 ml diH2O

   ➔ pH adjustment
   - provide 300 ml of 100 mM Na_2HPO_4 with 25 mM ethanolamine
   - add slowly about 250 ml of 100 mM $NaH_2PO_4 \cdot diH_2O$ with 25 mM ethanolamine until pH 8.5 is reached
3. Incubation Buffer and Wash Buffer I (PBST): 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.5 with 0.05 % Tween® 20.
4. Wash Buffer II (PBS): 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.5.

2.5 Equipment required

1. Cover slips (like Menzel BBAD02400600#AC or Marienfeld 0102242).
2. 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.
3. Centrifuge with slide holders or compressed nitrogen gas for drying slides.
4. Coplin jars (VWR 25457-006), Shandon™ Horizontal Staining Jars (Thermo Scientific #133) or similar for washing slides.

2.6 Protein concentration for spotting

NEXTERION® Slide H provides covalent attachment of proteins though amine groups of amino acids side chains on the protein surface. The coupling efficiency of the covalent chemistry depends on a number of factors, including pH, protein print concentration, and the nature of the protein itself.

A protein probe concentration ranging from 0.1 to 1 mg/ml is recommended to ensure sufficient protein loading and to enable reliable and consistent assay results.

2.7 Array printing

NEXTERION® Slide H is compatible with all microarray printing or spotting methods, including contact printing and piezo or ink-jet technologies.

Note: 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.

2.8 Protein immobilization

Print proteins at 50 % relative humidity and then place arrays in a slide humidity chamber (95 %) for 1 h or alternatively overnight at 75 % rel. humidity (this will ensure maximum coupling efficiency to surface).

2.9 Storage of printed slides

If you want to store printed arrays, please do so after printing/immobilization, but before washing/blocking. The printed protein arrays can be placed in a slide box and stored sealed at -20 °C and are at least stable for 6 months (depending on the stability of the printed proteins).

2.10 Washing and blocking

Because NEXTERION® Slide H has a reactive surface chemistry, off-feature or unspotted areas must be deactivated (blocked) before any other biomolecules are incubated with the surface.
Failure to block the surface can lead to the covalent attachment of assay molecules to the NEXTERION® Slide H surface, thus leading to high background. The slides should be blocked after printing as described below. Due to the low nonspecific binding characteristics of the surface the use of proteins in the blocking solution is not recommended, and actually discouraged. Do not use non-fat dry milk in the blocking or assay steps.

1. Submerge slides in the blocking solution (stipulated in the reagents required section) for 1 h to deactivate remaining functional groups. This can be performed in a clean 50 ml conical tube or other holder designed for microscope slides. Gentle agitation can be used.

Note that lab gloves may contain residues that can contaminate the surface and can lead to increased, non-uniform background. Avoid allowing residues from the gloves to flow onto the array.

2. Remove the slides from the blocking solution and rinse slides three times with Wash Buffer I (stipulated in the reagents required section) and one more time with diH_2O.
3. 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.

2.11 Assey conditions

The printed NEXTERION® Slide H slides are robust and compatible with most conditions encountered in protein-based assays. However, an incubation buffer comprised of phosphate buffered saline with 0.05 % Tween® 20 (also used as Wash Buffer I, see description under Reagents Required) is recommended. It is not advised to use non-fat dry milk containing buffers.

The Wash Buffer I described in the protocol above should be used between the various incubation steps in order to remove loosely bound material.

2.12 Target incubation

1. Dilute the labeled target in an appropriate amount of incubation buffer to allow full array coverage.
2. Pipette the target containing incubation buffer onto the array surface.
3. Carefully place a cover slip over the 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.

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 room temperature water bath. All incubations steps with labeled target should be carried out in the dark to avoid photo bleaching of the fluorescent dye.

2.13 Washing

Caution: Do not allow slides to dry between washes and protect from light whenever possible.

Note: The solutions recommended below for washing are a general guideline; alternative washes may be required depending on the application.

1. Remove the array from hybridization chamber, taking care not to disturb the cover slip.
2. Place the array into a slide rack and immerse in a dish containing Wash Buffer I. Plunge gently until the cover slip separates from the array.
3. Once the cover slips have been removed, place the arrays into a slide rack and immerse in a dish containing Wash Buffer I (PBST). Wash with shaking for 10 minutes. Repeat.
4. Wash in Wash Buffer II (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 any water stains on the slide surface.
6. Protect the array from light, dust, and handling until ready for scanning.