SCHOTT NEXTERION® Slide E

Protocol

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Protocol

1 DNA application

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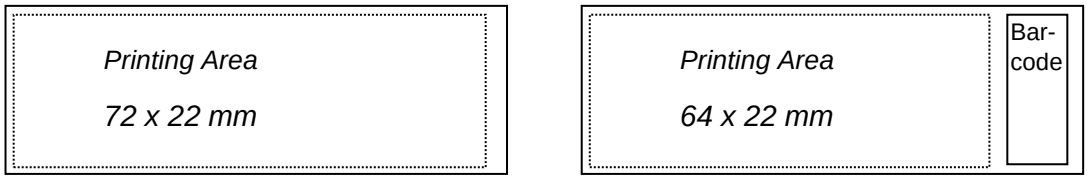
1.1 Introduction

NEXTERION® Slide 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.



1.2 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 scriber 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.

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1.3 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.

1.4 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

1.5 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.

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1.6 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® 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_2O.
   e. Rinse 1 x 1 min in diH2O at room temperature.
2. Proceed to Blocking immediately

1.7 Blocking

1. Block the slides with NEXTERION® 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® 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_2O at room temperature.
2. Dry the NEXTERION® 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.
3. Proceed to hybridization.

1.8 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.
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.

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1.9 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 laser and filter set of the scanner is compatible with the fluorescent labeling of the probe molecules.

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1.10. 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_2O 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_2O to 1000 ml	
	100 mM KCl	100 ml 1 M KCl stock solution, add diH_2O to 1000 ml	1 M KCl: dissolve 74,56 g KCl in 1000 ml diH_2O
Blocking Solution	Block E, diH_2O, HCl	Prepare as indicated on the Block E bottle	After addition of diH_2O 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_2O 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_2O, adjust pH to 7.0 with 10 N NaOH, add diH_2O to 1000 ml
	2x SSC	25 ml 20 x SSC, add diH_2O to 250 ml	
	0.2x SSC	2.5 ml 20 x SSC, add diH_2O to 250 ml	10% SDS: dissolve 10 g sodium dodecyl sulphate in 100 ml diH2O

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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® Spot is recommended especially when spotting oligonucleotides.

For Ring-And-Pin systems and for non-contact printing systems, a lower concentration of NEXTERION® 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.

Compatible Spotting Buffers for Slide E	
First Choice	NEXTERION® Spot[1]
Phosphate Based Buffers	• 50 to 300 mM sodium phosphate buffer pH 8.0 to 9.0[1] • 300 mM phosphate buffer + 0.005 % Tween® 20 + 0.001 % sarkosyl • 150 mM phosphate buffer + 0.001 % Tween® 20
SSC Based Buffer	• 3 x SSC
Low Evaporation Buffers	• NEXTERION® Spot LE (first choice) • 3 x SSC + 1.5 M betaine

1 Detergents can be added to increase spot size: SDS, sarkosyl, Tween® 20, Triton® X-100, Cetyl-trimethyl-ammoniumbromide. Final concentrations need to be determined empirically, usual concentration range is between 0.001 % and 0.05 % (final concentration).

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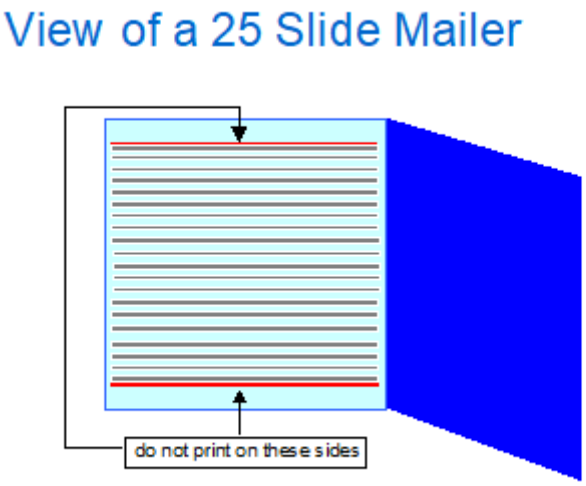
Can I print on both sides of the slides?

NEXTERION® Slide E is coated on both sides and both sides can be used as a printing side. However, due to the possibility of outgassing of material from the slide box it is advisable to print on the side facing away from the box for the terminal slides. The drawing illustrates this in principle for slides of a 25 slide mailer box.



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.

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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).

Unmodified oligonucleotides and unmodified PCR products	All probe types dissolved in a betaine containing spotting solution
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.

Storage spottled slides:

There are two recommended ways to store the printed slides:

1. Store the slides directly after spotting and any immobilization steps (i.e. humid 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.

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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.

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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_2O after hybridization.

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 per 5 Slides.

Product Ordering Guide:

Product	Item-No	Quantity	Description
NEXTERION® Slide E	1064016	25/pack	Barcoded Epoxy coated slides
NEXTERION® Slide E	1066643	25/pack	Non-barcoded Epoxy coated slides
Slide E Evaluation Kit	1117570	NEXTERION® Slide E 10/pack; NEXTERION® Spot 10ml; NEXTERION® Spot LE 10 ml; NEXTERION® Spot modified 10 ml; NEXTERION® Block E 100 ml; NEXTERION® Hyb 10 ml Probe Oligonucleotide 2 nmol Target Oligonucleotide 3 pmol	Non-barcoded Epoxy coated slides with test olinucleoptides and optimized reagents for evaluatios
NEXTERION® Spot	1066029	100 ml	Recommended printing buffer
NEXTERION® Block E	1066069 1066071	100 ml 1000 ml	Recommended chemical blocking reagent
NEXTERION® Hyb	1066075	100 ml	Recommended hybridization buffer
Epoxy Slide Oligo Processing Kit	1209009	NEXTERION® Oligo Pre-Hyb 500ml; NEXTERION® Oligo Hyb 10 ml; NEXTERION® Oligo Wash A 1000ml; NEXTERION® Oligo Wash B 100ml;	Pre-prepared reagents for chemical deactivation, hybridization and washing of 25 printed epoxysilane coated slides

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2 Protein application

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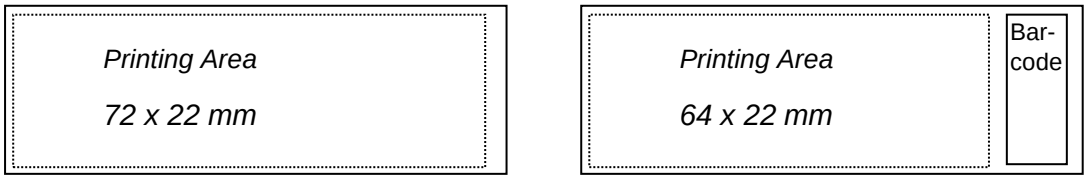
2.1 Introduction

Technical Instructions for Spotting Protein Microarrays

PRODUCT OVERVIEW

NEXTERION® Slide 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 excellent dimensional tolerances. The slide has a very low thickness deviation, an ultra-flat surface, and an extremely low inherent fluorescence. NEXTERION® Slide E was developed to provide the opportunity for fast and efficient coupling of proteins onto activated glass slides. The epoxy surface coating reacts with all nucleophilic groups provided by amino acid side chains (NH_2-, SH-, OH-) immediately and irreversible to form covalent bonds. 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.



2.2 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.

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

2.4 Reagent required

1. Protein Print Buffer: PBS (137 mM NaCl, 9 mM KOH, 11.3 mM NaH2PO4) pH 7.0 - 8.0 (a higher pH improve the immobilization but may interfere with protein stability)
2. Blocking Buffer: 1 % BSA in PBST (alternatively, 3 % nonfat milk in PBST can be used)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.
3. Wash Buffer: PBST (PBS with 0.05 % (v/v) Tween® 20 pH 7.4)
4. Rinse Solution: PBST

2.5 Equipment required

1. Humidified hybridization chamber (like GeneMachines HybChamber) or place a 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.
2. Centrifuge with slide holders or compressed nitrogen gas for drying slides.
3. Cover slips (like PGC Scientific 44-596).
4. Coplin jars (VWR 25457-006) or slide dish and rack combo (Fisher 900200) for washing slides.

2.6 Array printing

Print proteins at a final concentration of 0.05 - 0.2 mg/ml in the print buffer.

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

2.7 Protein immobilization

Print proteins at 50 % relative humidity and then place arrays in a humidity chamber at room temperature 1 hour.

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2.8 Blocking

Block slides for one hour in blocking solution at room temperature with slow shaking.

2.9 Assay conditions

For protein incubation steps on the printed slide, we have found that PBST works well as a dilution buffer for the target solutions. The blocking buffer can also be used if an increase in nonspecific binding is observed.

2.10 Target incubation

1. Dilute the labeled target sample in an appropriate volume of incubation or blocking buffer to cover the whole array.
2. Incubate with target solution for 1 hour.
3. Remove the coverslip and place the arrays into a slide rack. Immerse in a dish containing PBST. Wash with shaking for 10 minutes. Repeat.
4. Wash in 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 water stains on the slide surface.
6. Protect the array from light, dust, and abrasion of the array surface, until ready for scanning.

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