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e-*Surf* Protein Spotting Kit

Protocol information

Description

e-Surf Glass Slides are designed to covalently immobilize protein for microarrays . The coupling to the surface takes place at pH 8-9 in a humid environment following printing.
e-Surf Glass Slides contain reactive groups which are moisture sensitive and must be stored desiccated.
Both sides of each glass slide are activated for immobilization.

April 2005

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TABLE OF CONTENTS

For Laboratory use only
Store desiccated at room temperature
Consistent results are obtained by precisely following the instructions below.

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Appendix 1: Read carefully before planning any experiment with *e-Surf Glass Slides*.

General

e-Surf Activated Slides are compatible with any system that can accept a slide with the following dimensions: 25 mm width 75 mm × length × 1 mm thickness. Fluorescent labelled targets are most commonly applied, and can be monitored by scanning.
e-Surf coating has been designed to specifically face protein microarray challenges. They provide high binding capacity together with good control of ligand orientation and extremely low fluorescence background.
General protocol is given on page 7 of this manual.

Coating

The e-Surf coating covers both sides of the 25 mm × 75 mm slide.
e-Surf Activated Slides are obtained by adsorption on glass of a hydrophilic polymer containing N,N-acryloyloxysuccinimide (NAS), the reactive group that is able to bind amino modified DNA and primary amines of lysines and arginines in proteins.

Stability

e-Surf Activated Slides are stable for 10 months in it's original heat sealed, desiccated packaging. Once the original packaging has been opened, any unused slides must be resealed and stored desiccated in the original packaging or any sealable bag.
e-Surf Activated Slides should be printed below 50% relative humidity. The recommended range is 30-45% relative humidity. At 50% relative humidity, a 6 hour printing run is possible. Use lower humidity levels for longer print times.

Printing and coupling

The print buffer ***(150 mM sodium phosphate 0,01%TritonX100 pH 8.5)*** is designed specifically for use with ***e-Surf slides***. This pH (8.5) allows maximum binding of the amine to the surface. Acceptable sodium phosphate concentrations are 50-150 mM at pH 8-9. Increasing molarity of printing buffer may help in reducing the spot size.
Additives such as ***DMSO, PEG, or glycerol***, normally used to prevent drying of printed spots decrease binding and/or destroy spot morphology. The nature of the ***e-Surf Activated Slides*** does not require that the printed spots remain solubilized for effective immobilization.
Any amine containing primers or buffers should be avoided in the spot solution.
Binding of amine-labelled DNA or proteins to the slide surface occurs through a thermo chemical reaction. The saturated NaCl solution creates a 75% relative humidity environment that provides sufficient moisture for this reaction to proceed. If the printed slides are exposed to 100% relative humidity, the spots may enlarge or distort.

Amine modification

Oligonucleotides must be synthesized with an amine modified attached at either the 5' or 3'-end. PCR products are prepared by including a 5'-amine-modified primer in the amplification reaction. Primers labelled at the 3'-end will also function in PCR, but they are more expensive to synthesize and less commonly applied.

Quality control

Each lot of *e-Surf Activated Slides* is tested for the ability to bind reproducibly a standard quantity of amine-modified oligonucleotides and hybridise a standard quantity of Cy3 labelled target.

Description of Hybridisation procedure.
A 23 mer amino modified oligo is deposited onto glass surface: printing concentration is 10µM and print buffer is 150 mM phosphate buffer pH 8,5. Four sub arrays of 3 x 3 spots each are prepared to check entire glass surface. After an overnight incubation in humid environment, residual reactive groups are blocked using 50 mM ethanolamine and Tris 0,1M pH 9 for 15 minutes. To get the slide ready for hybridisation reaction the slide is first immersed in 4x SSC containing 0,1% SDS at 50°C for 15 minutes and than washed with water and air dried. A fluorescent oligonucleotide, complementary to that immobilized on the slide, diluted to 1µM in 2x SSC, 0,1%SDS and 0,2 mg/ml BSA is than added to the slide under a cover slip. Hybridisation takes place in humid chamber at 65°C for 2 h. After washing with 2 x SSC 0,1%SDS at 65°C and than with decreasing concentrations of SSC at room temperature, the slide is spin dried and scanned. Instrument software converts scanned images into spots and background fluorescence numbers. The ratio between the two represents the result of the assay.

a) Wash slide with 1x PBS for 5 minutes on the shaker. *Use at least 10 ml per slide*. Use a squirt bottle containing water to rinse.
k) Spin dry the slides.
l) Scan the slides

Ensure that slides are completely dry before scanning.

AVAILABILITY

Description	*Quantity*	*Product N°*
e-Surf Glass Slides	4 x 5 slides	MA0110
Protein Print Buffer	100 ml	BUF0140
Protein Blocking Buffer	250 ml	BUF0150
Protein Binding Buffer	100 ml	BUF0160
Wash Solution (10 x)	250 ml	BUF0170

Reagents and Materials Required

1. For printing and coupling Proteins

a) Print Buffer : 0.1 M sodium phosphate, 0.3 M NaCl 0,01% Triton x100 pH 7.2
b) Saturated NaCl humidification chamber: Add as much solid NaCl to water as needed to form a 1 cm deep slurry in the bottom of a plastic container with an airtight lid. This forms a chamber with a relative humidity of approximately 75%.
c) Slide racks

2. For post-coupling and binding:

a) Blocking Buffer: 50 mM sodium phosphate, 2.0% w/v Bovine Serum Albumin pH 7.2 For peptides use 50 mM ethanolamine, 0.1 M Tris, pH 9.0
b) Protein binding buffer (specimen and conjugate) : 0.1M Tris/HCl pH 8.0, 0.1 M NaCl, 0.02% w/v Tween 20, 1.0% w/v Bovine Serum Albumin.
c) 10 x Wash solution: 0.5M Tris 2.5M NaCl, 0.5% w/v Tween 20,pH 9.0
d) Shaker
e) Microcentrifuge
f) Centrifuge with microplate carriers
g) Hot plate
h) Humidified incubator or water bath
i) Binding chambers
j) Cover slip

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Immobilization Protocols

1. Preparation of Protein printing solution

a) Prepare antibodies to a final concentration of 0,5-1 mg/ml in Print Buffer (0.1 M sodium phosphate, 0.3 M NaCl 0,01% Triton X100 pH 7.2).
b) Protein challenge and optimal buffer may vary from assay to assay and therefore it should be determined by each final user.

2. Printing and coupling Proteins

a) Remove the slides from the sealed package. *Unused slides should be stored inside foil pouch with desiccant.*
b) Print protein solution on activated slides to form microarrays.
c) Place printed slides in a slide storage box.
d) Set uncovered storage box in the saturated NaCl chamber.
e) Seal chamber and allow to incubate at room temperature. *Overnight incubation has shown the best results. Incubate for a minimum of 2 hours-maximum of 72 hours.*
f) Store coupled slides at ambient condition until use. For long term storage, keeping slides desiccated is recommended.

3. Post-coupling processing

a) Place the slides in a slide rack and block residual reactive groups using 50 mM sodium phosphate, 2.0% w/v Bovine Serum Albumin pH 7.2 (Blocking buffer) for 1 hour. *Use at least 10 ml per slide.*
b) Discard the blocking solution.
c) Rinse the slides twice with bidistilled water. *Use at least 10 ml per slide.*

Do not allow slides to dry prior to centrifugation.

i) Place slides in the rack and centrifuge at 800 rpm for 3 minutes with microplate carriers.

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Binding Protocols

1. Specimen collection and preparation

Specimens showing particulate matter or turbidity should be centrifuged (relative centrifugal force of 1000-1200g for 5-15 min) before testing.
Before testing, totally thaw deep-frozen specimens, bring to room temperature (15-30°C), mix well and centrifuge (relative centrifugal force of 1000-1200g for 5-15 min) where appropriate.

2. Assay procedure

a) If required dilute specimen in binding buffer.
b) Immediately apply specimen to microarray prepared above. *Add 2.5 µl of target per cm^2 of cover slip.*
c) Place slides in binding chambers and transfer to humidified incubator or a water bath at the appropriate temperature for one hour.
d) Remove slides from binding chamber and use a squirt bottle containing water to rinse and remove the cover slip.
e) Wash slide with 1 x wash solution for 5 minutes on the shaker. *Use at least 10 ml per slide.*
f) Spin the slides to remove excess liquid.
g) Prepare conjugate (Dye/Protein ratio of 1) to a final concentration of 10-50 µg/ml in binding buffer.
h) Immediately apply conjugate to microarray prepared above. *Add 2.5 µl of target per cm^2 of cover slip.*
i) Place slides in binding chambers and transfer to humidified incubator or a water bath at the appropriate temperature for one hour.
j) Remove slides from binding chamber and use a squirt bottle containing water to rinse and remove the cover slip.