**LifeLineLab s.r.l.**
Via Nicaragua, 12/14
00040 Pomezia (RM) Italy
Tel. +39 06  916 016 28
Fax +39 06 916 12 477
info@lifelinelab.com
www.lifelinelab.com

LifeLineLab

# e-Surf Nucleic Acids Spotting Kit


# Protocol information

Description

*e-Surf* Glass Slides are designed to covalently immobilize amino-modified DNA 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**

LifeLineLab

# TABLE OF CONTENTS



For Laboratory use only

Store desiccated at room temperature

Consistent results are obtained by precisely following the instructions below.



LifeLineLab

## 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.

*3. Wash and scan*

a) Remove slides from hybridization chamber and use a squirt bottle containing room temperature 4X SSC to rinse and remove the cover slip. Place slides in rack and wash with:
   - hybridization-temperature 2X SSC / 0.1% SDS for 5 minutes. Discard the solution. Repeat
   - room temperature 0.2X SSC for 1 minute. Discard the solution.
   - room temperature 0.1X SSC for 1 minute. Discard the solution.

b) Spin dry the slides.

c) 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 |
| 2 x Print Buffer | 100 ml | BUF0100 |
| 4 x Blocking Solution | 250 ml | BUF0110 |
| 10% SDS | 100 ml | BUF0120 |
| 20 x SCC | 250 ml | BUF0130 |



LifeLineLab

# Reagents and Materials Required

*1. For printing and coupling DNA probes:*

a) 2x Print Buffer: 300 mM sodium phosphate 0,02% Triton x100, pH 8.5
b) PCR purification kit or desalting columns
c) 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%.
d) Slide racks

*2. For post-coupling and hybridization:*

*a)* 4x Blocking Solution: 200 mM ethanolamine, 0.4 M Tris, pH 9.0
b) Post-coupling wash solutions: 4x SSC / 0.1% SDS
c) Labeled targets in suggested hybridization buffers.
d) *For oligonucleotide (oligo) arrays*: 2x SSC, 0.1% SDS, 0.2 mg/mL Bovine serum albumin.
e) *For PCR product arrays:* 50% formamide, 5x SSC, 0.1% SDS, 0.2 mg/mL Bovine serum albumin.
f) Post-hybridization wash solutions:
   4x SSC
   2x SSC / 0.1% SDS
   0.2x SSC
   0.1x SSC
g) Shaker
h) Microcentrifuge
i) Centrifuge with microplate carriers
j) Hot plate
k) Humidified incubator or water bath
l) Hybridization chambers
m) Cover slip

LifeLineLab

# Immobilization Protocols

## *1. Preparation of amino-containing DNA probes*

a) Custom synthesized 3' or 5' amino-modified oligos should be HPLC or FPLC grade. Use desalting columns to remove amine contaminants. *Amine contaminants (e.g., ammonia and Tris) in the DNA probe preparations will decrease the immobilization efficiency.*

*b)* Amine-PCR products can be prepared by using 5'-amine modified primer on the strand that would be complementary to the labeled target. PCR products can be purified by ethanol precipitation (run the procedure twice), or with a commercial PCR Purification Kit (use 10 mM sodium phosphate buffer at pH 7.0-8.5 in the final elution step. *Dilute 2x Print Buffer 1:30 with water. Do not use Tris).*

c) The purified DNA probes should be stored at –20° C.

## *2. Preparation of DNA printing solution*

a) Prepare desalted amine-oligo to a final concentration of 10-15 pmoles/µL in 1x Printing Buffer (150 mM sodium phosphate, 0,01% Triton x100, pH 8.5

b) For aminated cDNA (0.1 to 1 kb), a concentration of 100-500 ng/µL DNA in 1x Printing Buffer is recommended.

## *3. Printing and coupling DNA*

a) Remove the slides from the sealed package. *Unused slides should be stored inside foil pouch with desiccant.*

b) Print DNA 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 4 hours-maximum of 72 hours.*

f) Store coupled slides at ambient condition until use. For long term storage, keeping slides desiccated is recommended.



LifeLineLab

# Hybridization Protocols

## *1. Post-coupling processing*

a) Place the slides in a slide rack and block residual reactive groups using pre-warmed 1x Blocking Solution at 50°C for 15 minutes (extend to 30 minutes if not warm. Do not exceed one hour).

b) Discard the blocking solution.

c) Rinse the slides twice with water.

*d)* Wash slide with 4x SSC / 0.1% SDS (pre-warmed to 50°C) for 15 to 60 minutes on the shaker. *Use at least 10 ml per slide.*

e) Discard wash solution and rinse briefly with water.

*f) For oligo arrays, skip to "h"*

g) For double-stranded DNA arrays, place slides into a boiling water bath for 2 minutes.

h) Rinse the slides twice with water.

**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.

## *2. Hybridization*

*a)* Prepare purified, labeled cDNA or oligo target in the hybridization buffer.

b) Heat the hybridization mixture containing double stranded DNA in a boiling water bath for 2 minutes.

c) *For oligo arrays skip to "e".*

d) Spin in microcentrifuge for 1 minute to cool the mixture.

e) Immediately apply target to microarray prepared above. *Add 2.5 µl of target per $cm^2$ of cover slip.*

f) Place slides in hybridization chambers.

g) Transfer hybridization chambers to humidified incubator or a water bath at the appropriate temperature for 4-16 hours.