

Technical Instructions for EPOXY microarrays

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I. INTRODUCTION

Epoxy Microarray Slides are manufactured mostly using the highest quality glass, which has an ultra-flat surface and extremely low inherent fluorescence. The epoxy surface coating allows efficient covalent and directed binding of molecules, e.g. synthetically fabricated oligonucleotides and/or PCR-products. This includes amino modified molecules, though modification is not required. PCR-products or oligonucleotides react instantly with the epoxy modified glass surface to form a covalent bond. The special cleaning and chemical coating procedures favor the generation of high-quality microarrays. The density of epoxy groups on the surface remains constant all over the slides and is adjusted to yield optimal binding.

II. GENERAL PRECAUTIONS

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.

1. Refer to manufacturer supplied Material Safety and Data Sheets (MSDS) for proper handling and disposal of the slides and all chemicals.
2. QInstruments buffers and solutions are for research use only, not for in vitro diagnostic use.

III. REAGENTS REQUIRED

For the spotting and binding of DNA-probes onto Epoxy surface coating Slides we recommend the optimized QInstruments spotting solution **Qlspot** (2x).

For blocking arrays printed on Epoxy Slides we recommend our 4x **Qlprocess**, which should be diluted 1:4 with water prior to the blocking process.

For majority of hybridization applications and wash steps we recommend our hybridization buffer **Qlhyb** and wash buffers **Qlwash I, II & III** (10x).

1. Deionized water (dH₂O) - at least 18.2 Megohms-cm resistance is recommended
2. 2x spotting solution **Qlspot** or alternatively 3x SSC or alternatively 1.5 M betaine in 3x SSC
3. 4x blocking solution **Qlprocess** (Dilute 100 ml 4x blocking solution with 300 ml dH₂O and 80 µl of 37 % HCl to get 1x solution) or alternatively 50 mM ethanolamine, 0.1% SDS (add freshly before use) in 0.1 M Tris, pH 9.0
4. Hybridization buffer **Qlhyb** or alternatively 3-5x SSC + 0.1 % SDS
5. Washing buffer **Qlwash I, II & III** (10x)
or alternatively:
 - a) Saline Sodium Citrate (20x SSC) – Ambion #9673
 - b) Sodium Dodecyl Sulfate (SDS) – Fisher #BP166-500 or alternatively 10 % SDS solution for washing (10 g dodecyl sulfate sodium salt in 100 ml dH₂O, dissolve at room temperature)
6. 0.1% Triton-X100 (1ml Triton-X100 in 1000 ml dH₂O, dissolve by heating up to 60°C)
7. HCl solution (Add 100 µl 37% HCl to 1000 ml dH₂O)
8. KCl solution (Dilute 100 ml 1 M KCl stock solution to 1000 ml dH₂O)



IV. EQUIPMENT REQUIRED

1. QInstruments incubation chamber **IHC1** for 12 slides or 3 microtiter plates
2. QInstruments hybridization system **PALMhyb professional** or **HC4 professional** or alternatively heat block - capable of heating to 95°C
3. Heated water bath
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



PALMhyb I

V. ARRAY PRINTING

The probes should be dissolved in water and diluted 1:1 with 2x **Qlspot** spotting solution to yield a 1x spotting solution. Therefore, the DNA-molecules have to be adjusted to the **double** of the final spotting concentration according to the recommended values in the following table. For quantitative validations, e.g. gene expression profiles, the DNA-molecules to be immobilized must always be available in excess.

1. Add detergents to **Qlspot** (2x) spotting solution to improve the performance:

Recommended solutions for different applications	
Characteristics	Add detergents to improve the performance
Create relatively small spots for majority of applications	-----
Good results using the Ring-And-Pin Technology	0.02 % Hexadecyltrimethylammonium bromide (C ₁₉ H ₄₂ BrN) <i>Merck, #814119</i>
Create bigger spots with pin and pipetting spotting systems	0.02 % Triton X-100 <i>Merck, #108603</i>
Amino surface coating	Mix equal amounts of oligonucleotide probe or PCR product and 50% DMSO to obtain a minimum final probe concentration of 20 µM for oligonucleotides, or 0.3 mg/ml for PCR products in 25% DMSO. Note: For smaller spot sizes, 3xSSC can be used as a printing buffer.

2. Resuspend cDNAs at 0.20-1.0 µg/µl and oligonucleotides at 20-40 µM in distilled H₂O.
3. Transfer 5.0 µl of each resuspended DNA sample into a 96- or 384-well microtiter plate.
4. Add 5.0 µl per well of **Qlspot** (2x) to obtain a recommended final probe concentration according to the following table:

Recommended final spotting concentrations		
Surface coating	Oligonucleotides	PCR products, cDNA
Epoxy	10 to 20 µM	0.1 to 0.5 µM (0.2 to 1 µg/µl)

5. Mix the samples thoroughly by pipetting up and down 10 times and centrifuge the plate for 5 min.
6. Setup the arrayer according to the manufacturer's recommendations.
7. Print the DNA samples onto Microarray substrate (optimal environment is 20 °C an 40-50 % relative humidity)

Notes:

- The results always depend on your equipment, your probes and your experimental conditions.
- Do not use any spotting solution containing primary amino-groups like Tris for Epoxy surface coating slides!
- The spot morphology and fluorescence uniformity can be modified by the chemical composition and concentration of the spotting solution. The optimal concentration mainly depends on the spotting technology and always needs to be adjusted to the experimental setup.
- **Qlspot** spotting solution is recommended for majority of applications.

- For Ring-And-Pin systems and for pipetting systems based on the capillary principle we also recommend trying out lower concentrations of **QIspot** spotting solution.
- When using PCR-products containing amino-functional primers, the primer should be separated prior to spotting (e.g. spin columns, microplates for primer removal).
- Use of the 2x **QIspot** spotting solution is advantageous especially when spotting oligonucleotides up to a length of 50 bases.
- Alternatively 3X SSC or 3X SSC containing 1.5 M betaine can be used as spotting buffers.
- DNA-probes in **QIspot** spotting solution can be stored at -20°C until spotting. If the probe solution shows a white precipitation prior to spotting, heat the probes to $50 - 80^{\circ}\text{C}$ for 2 min and avoid any change of concentration by condensation.
- 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.
- If you use a diamond scribe to mark the boundaries of the array, this produces small glass fragments, which may get trapped under the coverslip and damage parts of the array.

VI. DNA IMMOBILIZATION

1. Incubate printed microarray slides in humidity chamber (e.g. QInstruments incubation chamber **IHC1** for 12 slides) consisting of dH_2O for:
 - a) 30 min for binding of amine-modified oligonucleotides or
 - b) 30 min for binding of amine-modified PCR products, followed by an exposure to 60°C for 30 min.

Notes:

- When using unmodified probes, incubate the printed microarray slides at 120°C for 30 min in addition to the above treatment.
- For betaine-containing spotting solutions (i.e. 1.5 M betaine in 3X SSC), the microarrays should be incubated at 60° to 120°C for at least 60 min for efficient immobilization of oligonucleotides or PCR-Products.

2. Proceed to Washing

Notes:

- After spotting and immobilization, the arrays can be used immediately or stored under dry and dark conditions at room temperature.
- The washing steps after immobilization should not be carried out until immediately prior to hybridization.

VII. 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-X100 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) Rinse 1 x 1 min in dH_2O at room temperature

Notes:

- If you have spotted PCR probes, an additional denaturing step after KCl rinse is essential. Boil slides 1 x 3 min in dH_2O at $95 - 100^{\circ}\text{C}$.
- The volume of washing solution should be at least 250 ml for 5 slides.

2. Proceed to Blocking immediately

Note:

- Make sure that slides do not dry between washing steps and between washing and blocking.

VIII. BLOCKING

Failure to totally block the highly reactive **epoxy** groups will eventually result in covalent coupling of the labeled target to the slide surface leading to increased background signal. In addition to non-specific covalent attachment of substances, weak hydrophobic or electrostatic association of the labeled target with the slide surface might also occur. Both would increase the background fluorescence, but can be eliminated or substantially decreased through the use of **QIprocess**, thereby increasing signal to background ratio.

For blocking arrays printed on **Epoxy** Slides we recommend our 4x **QIprocess**, which should be diluted 1:4 with water prior to the blocking process.

To get 1x solution:	
Surface coating	
Epoxy	Dilute 100 ml 4x QIprocess Blocking Solution with 300 ml dH ₂ O and 80 µl of 37% HCl

1. Block the slides with QInstruments blocking solution **QIprocess** (4x) or alternatively with 50 mM ethanolamine, 0.1% SDS (add freshly before use) in 0.1 M Tris, pH 9.0 as follows:

- Incubate slides 1 x 15 min in 1x **QIprocess** blocking solution at 50°C and
- Rinse 1 x 1 min in dH₂O at room temperature.

Note:

- The volume of 1x **QIprocess** blocking solution should be at least 100 ml for 5 slides.

2. Dry the slides in an oil-free air or nitrogen stream or centrifuge (2 min at 150 to 200x g) to avoid any water stains on the slide surface.

3. Proceed to hybridization.



IX. HYBRIDIZATION

The hybridization of Microarray Slides can be done with the QInstruments **DiscoverSlip** technology + **PALMhyb professional**, or with 'cover slips' or 'gene frames' in QInstruments hybridization chamber **HC4** or alternatively in other commercially available hybridization chambers.

1. Labeling probe and Purify using Purification Kit.

2. Re-suspend the dried, fluorescent labeled target that will be applied to the array in **QIhyb** hybridization buffer. In case the target is already dissolved in a different buffer or in water, the sample can also be diluted in **QIhyb** to get at least 90% (v/v) in the final hybridization solution (mixture ratio sample:buffer = 1:9).

Recommended final sample concentrations	
Sample	
cDNA / Oligonucleotide and other DNA molecules	Dilute sample in QIhyb to get at least 90% (v/v) in the final hybridization solution, mixture ratio sample:buffer = 1:9

Notes:

- The amount of buffer depends on the desired target concentration and the size of hybridization chamber used.
- As an alternative to the QInstruments hybridization buffer, a buffer with 3-5x SSC + 0.1 % SDS can be used.

3. Denature the suspended 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.

Note:

- If the sample cannot be applied immediately after denaturation, then place it in a 42°C water-filled well of a heat block (e.g. QInstruments **HC4-S**).

4. Pipette the appropriate volume onto the array surface of a blocked slide under the cover slip and inside a **QInstruments hybridization chamber HC4**.

5. Hybridize the probe to the microarray under the appropriate conditions. QInstruments hybridization chamber **HC4** or temperature systems **HC4-S** or **HC4-S2** are recommended.

X. POST-HYBRIDIZATION WASHING

Wash away unbound labeled probe. QInstruments microarray wash buffers **QIwash I, II & III** (10x) are recommended.

To get 500 ml of 1x wash buffer:	
Wash buffer	
QIwash I	Dilute 50 ml 10x QIwash I with 450 ml dH ₂ O (<i>mix under agitation</i>)
QIwash II	Dilute 50 ml 10x QIwash II with 450 ml dH ₂ O (<i>mix under agitation</i>)
QIwash III	Dilute 50 ml 10x QIwash III with 450 ml dH ₂ O (<i>mix under agitation</i>)

Cautions:

- Do not allow slides to dry between washes, and protect from light as much as possible.
- Never wash the slides with dH₂O after hybridization.
- Make all wash steps with vigorous agitation.
- The solutions recommended below for washing are a general guideline; your application may require alternative stringency washes.

1. Place the microarray slides (max 10 pcs.) into a slide rack and immerse in a dish containing 500 ml **QIwash I**. Wash in the above solution 1 x 10 min at room temperature.
2. Wash 1 x 10 min in 500 ml **QIwash II** at room temperature.
3. Wash 1 x 10 min in 500 ml **QIwash III** at room temperature.

or alternatively Step 1 to 3:

- a) Place the array into a slide rack and immerse in a dish containing 2x SSC and 0.2% SDS. Wash in the above solution 1 x 10 min at room temperature.
- b) Wash 1 x 10 min in 2x SSC.
- c) Wash 1 x 10 min in 0.2x SSC at room temperature.

Notes:

- The volume of the washing solution should be at least 250 ml for 5 Slides.
4. Dry the microarray slides in an oil free air or nitrogen stream or by centrifugation at 2 min at 150 to 200x g 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.

XI. SCANNING

Scan the microarray to acquire a fluorescent image. Ensure that the laser and filter set of the scanner is compatible with the fluorescent labeling of the probe molecules.

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