

Buffers and Solutions for Microarraying

Microarray printing buffer Qlspot (2x)

Technical Instructions for spotting microarrays

I. INTRODUCTION

QInstruments printing buffer **Qlspot** (2x) is an advanced ionic and polymeric buffer system designed to increase the quality of microarray biochip fabrication by improving the surface properties of the DNA samples deposited by direct contact and non contact DNA microarray spotting technologies. It has been developed to ensure excellent spot morphology with optimal spot size, high signal intensities with reduced unspecific background and overall data reproducibility. The advanced mixture allows efficient covalent and directed binding of molecules, e.g. synthetically fabricated oligonucleotides and/or PCR-products.

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 all chemicals.
2. QInstruments buffer and solutions are for research use only, not for in vitro diagnostic use.

III. PRODUCT DESCRIPTION

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

Make your own decision by selecting the best spotting solution appropriate and optimize your experiments and your spotting setup. As an general alternative 3x SSC or 1.5 M betaine in 3x SSC can be used. For **Amino** surface coating Slides we recommend alternatively DMSO.

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.

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.

Recommended final probe concentrations		
Surface coating	Oligonucleotides	PCR products, cDNA
Epoxy	10 to 20 µM	0.1 to 0.5 µM (0.2 to 1 µg/µl)
Aldehyde	10 to 20 µM	0.1 to 0.5 µM (0.2 to 1 µg/µl)
Amino	2 to 20 µM	0.05 to 0.5 µM (0.1 to 1 µg/µl)

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IV. PROTOCOL

1. Resuspend cDNAs at 0.20-1.0 µg/µl and oligonucleotides at 20-40 µM in distilled H₂O.
2. Transfer 5.0 µl of each resuspended DNA sample into a 96- or 384-well microplate.
3. Add 5.0 µl per well of **Qlspot** (2x).
4. Mix the samples thoroughly by pipetting up and down 10 times und centrifuge the plate for 5 min.
5. Print the DNA samples onto Microarray substrate (*optimal environment is 20°C an 40-50 % relative humidity*)
6. Process the printed substrates for hybridization.

The use of the printing buffer **Qlspot** (2x) is described in detail in our 'Complete Protocol for Epoxy Slides', 'Complete Protocol for Aldehyde Slides' and 'Complete Protocol for Amino Slides'.

Notes:

- Do not use any spotting solution containing primary amino-groups like Tris for Epoxy and Aldehyde 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 to try out lower concentrations of **Qlspot** 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 **Qlspot** 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 **Qlspot** 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°C for 2 min and avoid any change of concentration by condensation.

Cautions:

- The results always depend on your equipment, your probes and your experimental conditions.
- 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.
- 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.

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