

Microarray blocking solution Q.process (4x)

Technical Instructions for blocking microarrays

I. INTRODUCTION

Q.Instruments **Q.process** (4x) is an optimized blocking solution designed to block **Epoxy** glass microarray surface chemistries prior to protein binding and nucleic acid hybridizations for ultra low background. It has been developed to ensure excellent spot morphology with optimal spot size, high signal intensities with reduced unspecific background and overall data reproducibility. Use of **Q.process** will increase the quality of microarrays prepared by all DNA printing technologies by providing uniform spreading of the sample on modified microarray slides.

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. Q.Instruments buffer and solutions are for research use only, not for in vitro diagnostic use.

III. PRODUCT DESCRIPTION

The blocking solution **Q.process** (4x) is easy to prepare and avoids working with unnecessarily hazardous chemicals or protein batches with varying quality (i.e. BSA, that is commonly used for blocking).

After spotting it is important to remove unbound DNA-molecules and buffer substances from the slides by extensive washing to avoid any interference with subsequent hybridization experiments. Consequently, we recommend to block the epoxy slides with our **Q.process** (4x) Blocking Solution before the microarray is hybridized.

The blocking solution **Q.process** (4x) contains molecules that rapidly react with residual covalent binding sites in both, the printed and unprinted areas of the slide surface. Failure to totally block reactive groups may result in non-specific binding of labeled target and probe molecules to the slide surface.

This potentially irreversible covalent coupling of the labeled target is the first component of the non-specific background problem. The second component is weak adhesive association (hydrophobic or electrostatic) of the labeled target with the slide surface. Both of these cause a fluorescent background, which can be eliminated or substantially decreased through the use of Q.Instruments Blocking Solution **Q.process** (4x). This results in a higher reproducibility and quality.

IV. BLOCKING SOLUTION FOR EPOXY SURFACE COATING SLIDES

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 **Q.process**, thereby increasing signal to background ratio.

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

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

The use of the epoxy blocking solution is described in detail in our '*Complete Protocol for Epoxy Slides*'.

Q.Instruments Buffers and Solutions for Microarraying

V. BLOCKING SOLUTION FOR ALDEHYDE SURFACE COATING SLIDES

For blocking arrays printed on **Aldehyde** Slides we recommend to treat the exposed aldehyde groups with sodium borohydride (NaBH_4) thereby reducing them to hydroxyl groups. The aldehyde blocking solution for approximately 5 slides contains:

- 1.0 g NaBH_4
- 300 ml 1x PBS (phosphate buffer silane)
- 100 ml 99% ethanol (to reduce bubbling)

The use of the aldehyde blocking solution is described in detail in our '*Complete Protocol for Aldehyde Slides*'.

VI. BLOCKING SOLUTION FOR AMINO SURFACE COATING SLIDES

After immobilization it is important to remove unbound DNA molecules and buffer substances from the **AMINO** slides by extensive washing to avoid any interference with subsequent hybridization experiments.

To avoid bleeding of the spots it is important to perform the pre-hybridization washing steps very quickly by moving the slides (slide holder) up and down in the rinsing solution rather than using a shaker. The blocking is either done by prehybridization with BSA (protocol A, blocking past washing) or by reaction of the NH_2 -groups with succinic anhydride (protocol B, blocking prior to washing).

Amino slide pre-hybridization solution (protocol A with pre-hybridization):

- 25 ml Q.Instruments **Q.hyb** hybridization buffer
- 25 ml dH_2O
- 500 mg BSA

(amount sufficient for up to 5 slides)

Amino slide blocking solution (protocol B with blocking step):

- 5 g succinic anhydride
- 315 ml n-methylpyrrolidone
- 35 ml 0.2 M sodium-borate pH 8 (Add sodium-borate freshly before use)

(amount sufficient for up to 20 slides)

The use of the amino blocking solution is described in detail in our '*Complete Protocol for Amino Slides*'.

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