MICROARRAY HYBRIDIZATION WASH PACK

ProductInformation

Product No. **M 2185**
Technical Bulletin No. MB-960
Storage Temperature RT

TECHNICAL BULLETIN

Product Description
Microarray Hybridization Wash Pack provides optimized blocking and wash solutions for microarray slides. The wash solutions have been validated with SigmaScreen™ Slides for Microarrays and other commercially available aminopropylsilane slides.

Precautions and Disclaimer
Microarray Hybridization Wash Pack is for laboratory use only. Not for drug, household or other uses.

Storage/Stability
Store the Microarray Hybridization Wash Pack at room temperature.

Reagents Provided

Product Name:	Product Code
Microarray Hybridization Blocking Buffer, 1 x 1L	M 1685
Microarray Post Hybridization Wash Buffer I, 2 x 1L	M 1810
Microarray Post Hybridization Wash Buffer II, 1 x 1L	M 1935
Microarray Post Hybridization Wash Buffer III, 1 x 1L	M 2060

Equipment and Reagents Required But Not Provided (Product codes have been given where appropriate)

Product Name:	Product Code
ArrayHyb™ Hybridization Buffer	A 7718
ArrayHyb™ LowTemp Hybridization Buffer	A 3095
Humid chamber	H 6644
Slide staining chambers/racks	S 6141
22 x 22 mm Coverslips	C 9802
Ethanol, 95+%	E 7148
20X Saline-sodium citrate (SSC) buffer	S 6639
Slide staining chambers/racks	S 6141
Orbital shaker (The Belly Dancer)	Z36,760-5

Preparation Instructions

1. **Printing of DNA Arrays on SigmaScreen Silane Slides.** The DNA to be arrayed should be prepared at a final concentration of 50-250 ng/µl in 3X SSC or other desired printing buffer. Print slides according to the arrayer manufacturer's protocol or a standard microarraying protocol for printing on aminopropylsilane slides. Store printed slides desiccated until ready for pre-processing and hybridization.

2. **3X SSC for DNA solutions.** Prepare 3X SSC by diluting 3 ml of 20X SSC (Product Code S 6639) with 17 ml of distilled, deionized water.

Procedure

A. Pretreatment of Slides for Hybridization

Slides must be pretreated after arraying targets, but prior to use in hybridization. The pretreatment procedure outlined below has been optimized for hybridizations using ArrayHyb or ArrayHyb LowTemp hybridization buffers. This step denatures the spotted, double stranded DNA to make it available for hybridization and washes away any excess spotted nucleic acid. If slides are pretreated using other procedures, overall signal and consistency may be compromised.
Multiple slides may be pretreated at the same time using this procedure. Do not write on the slide at this stage, as many writing sources are soluble in ethanol and can contribute to non-specific fluorescence. Slides that are not used immediately for hybridizations should be stored desiccated at 2-8 ºC.

1. Begin heating distilled, deionized water to 95-100 ºC in a clean container filled with sufficient water to cover slides in a slide staining rack.

2. Fill a humid chamber with approximately 50 ml of water prewarmed to approximately 65 ºC.

3. Invert arrayed slides (DNA side down) into the humid chamber and allow spots to rehydrate for approximately 5 seconds.

4. Snap-dry each arrayed slide (DNA side up) on a 95-100 ºC inverted heat block for approximately 5 seconds.

5. Place slides (DNA side up) in a second empty humid chamber.

6. Crosslink the DNA to the surface with 65 mJ of UV light at 254 nm. Place the slides in a slide staining rack.

7. Incubate the slides for approximately 2 minutes in a slide staining dish filled with Microarray Hybridization Blocking Buffer on an orbital shaker. This step removes the unbound nucleic acids from the arrays and helps block non-specific binding of nucleic acids.

8. Wash the slides by dipping the staining rack several times in a dish of distilled, deionized water. Fresh water should be used for each set of slides. This step removes the majority of the Microarray Hybridization Blocking Buffer from step 7.

9. Gently plunge the slide rack into the 95-100 ºC distilled, deionized water for 2 minutes.

10. Remove the slide rack from the water bath and rinse the slides by plunging the rack 10-20 times in 95+% ethanol.

11. Quickly transfer the slides to a centrifuge with a swinging bucket rotor for microtiter plates (place paper towels below rack to absorb liquid) and spin at 50-100 X *g* for 5-10 minutes.

12. Use the treated slides immediately or store in a slide box desiccated at 2-8 ºC.

B. Hybridization

This protocol is written for hybridization with ArrayHyb LowTemp Hybridization Buffer. If ArrayHyb Hybridization Buffer is utilized, the probe should be heated to 95-100 ºC (step 3) and the hybridization temperature should be increased to 65 ºC (step 6).

1. Equilibrate pretreated slides to room temperature and label each clearly with indelible ink.

2. Create a probe mixture by combining labeled nucleic acid and blocking agent(s), if desired (see below), in a microcentrifuge tube. Ethanol precipitate or use a speed-vac to dry the mixture. Resuspend the probe mixture pellet into the appropriate volume of ArrayHyb LowTemp hybridization buffer for the size cover slips being utilized. The optimal volume has been found to be 2.5-3 µl/cm^2 for ArrayHyb LowTemp (12.5-15 µl for 22 x 22 mm cover slips).

Common Blocking Agents	Final Concentration
Single stranded DNA	100 µg/ml
Poly dA	400 µg/ml
Yeast tRNA	200 µg/ml
CoT-1 DNA®	400 µg/ml

3. Heat the probe mixture at 60 ºC for 5 minutes.

4. Centrifuge the liquid to the bottom of the microcentrifuge tube and carefully pipette the hybridization solution onto a cover slip.

5. Slowly lower the slide (array side down) until surface tension allows the cover slip to be raised with the slide, taking care not to introduce bubbles.

6. Incubate the slides from 6 hours to overnight at 50 ºC in a humidity-controlled environment. This can be achieved by placing slides in an empty humid chamber placed in a shallow hybridization water bath; alternatively, hybridization may be carried out in one of several commercially available hybridization chambers immersed in a temperature controlled water bath.

C. Washing

Do not allow the slides to dry out at any point during this procedure. This can cause non-specifically bound probe to become permanently attached to the slide resulting in high backgrounds.

1. Remove the slides one at a time from the water bath, immerse the slides into a clean container filled with Wash Buffer I, and gently remove the cover slips. Place the slides into a slide rack/staining dish filled with Wash Buffer I and incubate for 5 minutes at room temperature with gentle mixing on an orbital shaker.

2. During this incubation, place 200 ml of Wash Buffer II in a clean staining dish containing a clean slide rack.

3. Quickly transfer the slides, one at a time, to the rack in Wash Buffer II. Shake off excess Wash Buffer I from each slide as it is transferred to the rack in Wash Buffer II.

4. Incubate the slides in Wash Buffer II for 5 minutes at room temperature on an orbital shaker.

5. During this incubation, place 200 ml of Wash Buffer III in a clean staining dish (no slide rack required).

6. Transfer the slide rack containing the slides to the staining dish containing Wash Buffer III and incubate for 5 minutes at room temperature on an orbital shaker.

7. After the third washing, quickly transfer the slides to a dry slide rack and place in a centrifuge equipped with a swinging bucket rotor for microtiter plates. Centrifuge at 50-100 X *g* for 5-10 minutes. Immediately remove the slides from the centrifuge and store in a light-proof slide box.

8. Store the slides protected from light and dust. Scan as soon as possible.

Troubleshooting Guide

Problem	Cause	Solution
Spots appear smeared or as comets	Recommended pretreatment procedure was not followed	When concentrated DNA (0.5 mg/ml) is spotted on the slide, only a fraction becomes bound to the surface. The remaining unbound DNA must be washed away. This is accomplished in the recommended pretreatment procedure by washing the slides in Blocking Buffer. This step removes unbound DNA from the spots and prevents the DNA from binding to the surrounding slide surfaces.
	DNA is too concentrated	Print with less concentrated DNA samples.
	UV treatment not effectively immobilizing DNA	Insure that a reliable UV light source (such as BioLink® BLX UV Crosslinker, Z37,537-3) is utilized.
Irregular spot morphology	Deformed spotting pins	Replace spotting pins.
	Poor printing of DNA	Dissolve the DNA in 3X SSC or another validated printing solution.
Low signal	Inefficient binding of nucleic acids to the slide during printing	DNA must be cleaned properly prior to spotting. Silica matrix columns, such as the GenElute™ PCR DNA Purification Kit (GEN-PCR), are recommended for purification of the DNA.
	Probe was not labeled efficiently or has been exposed to light	Check the probe for labeling efficiency. If poor label incorporation is observed, remake the probe. Protect labeled probes from exposure to light.
Background fluorescence	Drying of hybridization solution around edges of coverslip	Be sure to hybridize the slides in a humid chamber. The use of humid chambers will prevent this problem.
	Incomplete washing	Wash thoroughly using the recommended solutions and conditions.
	Probe was allowed to dry to the slide during transfer steps	Use extra care to prevent any drying of the probe solution on the slides.
	Dust has accumulated on the slide	Dust particles will show up as isolated spots of very high fluorescence. Protect the slides from general lab air as much as possible. Some dust may be removed by the use of compressed air to "dust" the slide prior to scanning.

Related Products

Product Name:	Product Code
ArrayHyb Hybridization Buffer	A 7718
ArrayHyb LowTemp Hybridization Buffer	A 3095
Aminoallyl cDNA Labeling Kit	AM-ALLYL
SigmaSpin™ Post Reaction Clean-up Columns	S 5059
GenElute PCR Purification Kit	GEN-PCR
SlideMoat™ Hybridization Incubator	Z38,067-9
BioLink BLX UV Crosslinker	Z37,537-3
Hybridization Water Bath	Z36,765-6
Single strand DNA for hybridization	D 7656
Poly $d(A)_n$	P 0887
Yeast tRNA	R 5636

References

1. Schena, M. et al., Parallel human genomic analysis: microarray-based expression monitoring of 1000 genes. Proc. Natl. Acad. Sci. USA, **93,** 10614-10619 (1996).

2. Schena, M. et al., Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science, **270,** 467-470 (1995).

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