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ProductInformation

ArrayHyb™ LowTemp Hybridization Buffer

Product No. **A 3095**
Technical Bulletin No. MB-920
Store at room temperature

TECHNICAL BULLETIN

Product Description

ArrayHyb™ LowTemp Hybridization Buffer is optimized for use with microarrays spotted on glass slides. Hybridization of fluorescent labeled nucleic acids using ArrayHyb LowTemp Hybridization Buffer consistently provides superior signal with minimal background when compared to standard and other commercially available hybridization buffers. In addition, this formamide-containing formulation allows for lower hybridization temperatures (42-50 °C) while maintaining high hybridization specificity. For instances in which formamide is undesirable, or if higher (>55 °C) hybridization temperatures are required, Sigma recommends ArrayHyb™ Hybridization Buffer (Product No. A 7718).

The ArrayHyb LowTemp formulation increases the rate of hybridization and can provide results for analytical purposes in as little as 2 hours. Approximately 60% of the maximum signal is achieved within 6 hours; however, the signal will continue to increase over time with no increase in background for greater sensitivity. This flexibility allows hybridizations to be tailored to meet specific requirements.

Precautions and Disclaimer

ArrayHyb LowTemp Hybridization Buffer is for laboratory use only. Not for drug, household or other uses.

Storage/Stability

Store ArrayHyb LowTemp Hybridization Buffer at room temperature. ArrayHyb LowTemp has a shelf life of 1 year upon receipt. If a small amount of precipitate forms, warm the solution to >30 °C until dissolved, invert to mix, and store at room temperature.

Reagents and Equipment Required But Not Provided
(Sigma product numbers have been given where appropriate)

Product Name	Product No.
Standard Microarray Spotting Solution	M 1435
SigmaScreen Coated Slides for Microarrays	S 7934
Humid Chamber	H 6644
Wheaton 20 slide staining dish	S 6141
22 x 22 mm Coverslips	C 9802
95% Ethanol	E 7148
Microarray Hybridization Wash Pack	M 2185
or	
10% Lauryl sulfate (SDS) Solution	L 4522
20X Saline-sodium citrate (SSC) buffer	S 6639

Preparation Instructions

The following solutions are components of the Microarray Hybridization Wash Pack (Prod. No. M 2185):

1. SDS solution (0.5%) for pre-treatment of slides prior to hybridization. Prepare the 0.5% SDS solution by diluting 50 ml of the 10% SDS solution (Product No. L 4522) with 950 ml of distilled, deionized water.

2. Wash Buffer 1 (1X SSC, 0.03% SDS). Prepare Wash Buffer 1 by combining 50 ml of 20X SSC (Product No. S 6639) with 3 ml of the 10% SDS solution. Dilute to 1 L with distilled, deionized water.

3. Wash Buffer 2 (0.2X SSC). Prepare Wash Buffer 2 by diluting 10 ml of 20X SSC to 1 L with distilled, deionized water.

4. Wash Buffer 3 (0.05X SSC). Prepare Wash Buffer 3 by diluting 2.5 ml of 20X SSC to 1 L with distilled, deionized water.

Procedure

A. Printing of DNA arrays on aminopropylsilane and poly-L-lysine slides

Double-stranded DNA should be purified and resuspended in water at a concentration of 0.1-1 μg/μl and then mixed with an equal volume of the Standard Microarray Spotting Solution. Oligonucleotides should be resuspended in water at a concentration of 100-150 μM and then mixed with an equal volume of the Standard Microarray Spotting Solution. Print slides according to the arrayer manufacturer's protocol or a standard microarraying printing protocol. Store printed slides desiccated at room temperature until ready for pre-treatment and hybridization.

B. Pre-treatment of Slides for Hybridization

Slides must be pre-treated after arraying the targets, but prior to use in hybridization.
The pre-treatment procedure outlined below was developed for SigmaScreen™ Coated Slides for Microarrays, which contain aminopropyltriethoxysilane-derived amine functional groups covalently attached to the slide surface. If a different type of slide has been utilized as a printing substrate, use a pre-treatment protocol optimized for that substrate. If the printed slides have already been pre-treated by similar procedures, proceed directly to Section B, Hybridization.
This protocol has been optimized for hybridizations using ArrayHyb LowTemp Hybridization Buffer. 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 pre-treated using other procedures, overall signal and consistency may be compromised.
Multiple slides may be pre-treated at the same time using this procedure. Do not write on the slide at this stage, as many inks are soluble in ethanol and can contribute to non-specific fluorescent signal. 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 humidity chamber with approximately 50 ml of water pre-warmed 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. UV crosslink the DNA to the slide surface with 65 mJ of 254 nm UV light. Place the slides in a slide staining rack.

7. Incubate the slides for approximately 2 minutes in a slide staining dish filled with 0.5% SDS solution 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 SDS 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 to 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 the rack to absorb liquid) and spin at 50-100 X *g* for 5 to 10 minutes.

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

C. Hybridization

1. Equilibrate pre-treated 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 dry the mixture with a speed-vac. 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 ArrayHyb LowTemp/probe mixture at 60 ºC for 5 minutes.

4. Centrifuge the contents 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.

D. 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 1, and gently remove the cover slips. Place the slides into a slide rack/staining dish filled with Wash Buffer 1 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 2 (0.2X SSC) 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 2. Shake off excess Wash Buffer 1 from each slide as it is transferred to the rack in Wash Buffer 2.

4. Incubate the slides in Wash Buffer 2 for 5 minutes at room temperature on an orbital shaker.

5. During this incubation, place 200 ml of Wash Buffer 3 (0.05X SSC) in a clean staining dish (no slide rack required).

6. Transfer the slide rack containing the slides to the staining dish containing Wash Buffer 3 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 to 10 minutes. Immediately remove the slides from the centrifuge and store in a light-proof slide box.

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

Troubleshooting Guide

Problem	Cause	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, are recommended for purification of the DNA.
	Hybridization temperature too high	Decrease hybridization temperature (typically by 5-10 °C) to achieve optimal hybridization signal.
	Probe was not labeled efficiently	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 at the edges of the cover slip	Be sure to hybridize the slides in a humidified chamber. The use of humid chambers will prevent this problem.
	Probe was allowed to dry to the slide during transfer to Wash Buffer 1	Use extra care to prevent any drying of probe solution on the slides.
	Dust has accumulated on the slide	Dust particles will show up as isolated spots of very high fluorescence. Protect 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.
Non-specific Hybridization Signal	Hybridization temperature too low	Increase hybridization temperature (typically by 5-10 °C) to eliminate non-specific hybridization signal.
Spots appear as streaks or comets	Recommended pre-treatment procedure was not followed	When concentrated DNA (0.5 mg/ml) is spotted on the slide, only a small percentage becomes bound to the surface. The remaining unbound DNA must be washed away. This is accomplished in the recommended pre-treatment procedure by washing the slides in 0.5% SDS solution. This step removes unbound DNA from the spots and prevents the DNA from binding to the surrounding slide surfaces.

Related Products

Product Name	Product No.
ArrayHyb™ Hybridization Buffer	A 7718
GenElute™ Mammalian Total RNA Kits	RTN-10 RTN-70 RTN-350
GenElute™ mRNA from Total RNA Kits	MRN-10 MRN-70
GenElute™ PCR Purification Kit	GEN-PCR
SigmaSpin™ Post-reaction Clean-up Columns	S 5059
0.1% Poly-L-Lysine	P 8920
3-Aminopropyltriethoxysilane	A 3648
Hybridization Water Bath	Z36,765-6
BioLink BLX UV Crosslinker	Z37,537-3
Belly-Dancer Orbital Shaker	Z36,760-5
Single-strand DNA for hybridization	D 7656
Poly $(dA)_n$	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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