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ProductInformation

SigmaScreen™ APS Coated Slides for Microarrays

Product Number **S 9936**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description
SigmaScreen APS Coated Slides for Microarrays are an optimal substrate for printing DNA microarrays. The slides are prepared by coating clean glass slides with aminopropylsilane resulting in covalent addition of amine functional groups to the slide surface. The availability of positively charged amine groups at the surface translates into high DNA binding and strong hybridization signals. The negatively charged phosphate backbone of DNA electrostatically attaches to the positively charged amines. Treatment with ultraviolet irradiation results in a more stable coupling.

Each lot of slides is rigorously quality tested under high standards for surface energy, coating uniformity, functional group density, autofluorescence, particulation, and array printing/ hybridization to ensure performance and lot-to-lot consistency.

Slide Dimensions: 75 mm X 25 mm X 1 mm

Precautions and Disclaimer
SigmaScreen APS Coated Slides for Microarrays are for laboratory use only, not for drug, household or other uses.

Storage/Stability
Store SigmaScreen APS Coated Slides for Microarrays at room temperature with desiccant. Use the slides in a clean environment. Particles can interfere with the printing (arraying) process and can lead to background signal during detection. In addition, slide handling should be minimized. Touching of the printing surface, except by the printer, should be avoided.

Reagents and Equipment Required but Not Provided (Product Codes have been given where appropriate)

Product Name	Product Code
ArrayHyb™ Low Temp Hybridization Buffer	A 3095
Standard Microarray Spotting Solution	M 1435
Humid Chamber	H 6644
Wheaton 20 slide staining dish	S 6141
22 x 22 mm Coverslips	C 9802
95% Ethanol	E 7148
Belly-Dancer/Hybridization Water Bath (115 V or 220/240 V)	Z36,762-1 Z36,764-8
BioLink BLX Crosslinker (115 V or 230 V)	Z37,537-3 Z37,538-1
Heat Block	

Preparation Instructions
Use the ready-to-use wash solutions from the Microarray Hybridization Wash Pack (Product Code M 2185) or prepare solutions according to Table 1.

Table 1.
Wash solutions

		Wash Buffers		
	0.5% SDS Solution	**1** 1x SSC, 0.03% SDS	**2** 0.2x SSC	**3** 0.05x SSC
10% SDS (L 4522)	50 ml	3 ml	---	---
20x SSC (S 6639)	---	50 ml	10 ml	2.5 ml
H_2O	to 1L	to 1L	to 1L	to 1L

Procedure
A. DNA Printing

Dilute double-stranded DNA (0.1-1 µg/µl in water) or oligonucleotide (100-150 µM in water) samples with an equal volume of the Standard Microarray Spotting Solution. Print slides according to arrayer manufacturer's or standard protocol. Store printed slides desiccated at room temperature until ready for pre-hybridization treatment and hybridization.

B. Post Printing/Pre-Hybridization Treatment

Slides must be pre-treated prior to hybridization after printing DNA or oligonucleotide samples. The following procedure has been optimized for hybridizations using ArrayHyb™ LowTemp Hybridization Buffer. Overall signal and consistency may be compromised if slides are pre-treated using other procedures. This procedure denatures spotted, double-stranded DNA, making it available for hybridization, while eluting any non-crosslinked nucleic acid. Multiple slides may be pre-treated simultaneously. Do not write on the slide at this stage; many inks are soluble in ethanol and may contribute to fluorescent backgrounds.

1. Heat enough distilled, deionized water to 95-100 °C to cover the slides in a slide staining rack.

2. Fill a humid chamber (H 6644) with approximately 50 ml of water and warm to 65 °C in a water bath. Other chambers may require more or less volume. The chamber bottom must be covered with water to facilitate equal hydration of the slides.

3. Place the arrayed slides, DNA side down, over the humid chamber. Allow spots to hydrate for approximately 5 seconds.

4. Dry the arrayed slide (DNA side up) on a 95-100 °C surface/heat block for approximately 5 seconds.

5. UV crosslink the DNA with the slide surface by placing slides, DNA side, up on a rack (an empty humid chamber) and supply 65 mJ of 254 nm irradiation.

6. Place slides in a slide 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. Rinse the slides by dipping the slide rack several times in a dish of fresh distilled, deionized water.

9. Gently plunge the slide rack into 95-100 °C distilled, deionized water for 2 minutes.
   Note: This step is not necessary for single-stranded oligonucleotides.

10. Rinse the slides by plunging the slide rack several times in a staining dish filled with 95+% ethanol.

11. Quickly transfer the slides to a centrifuge equipped with a swinging bucket rotor for multiwell 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 °C.

C. Hybridization

1. Equilibrate pre-treated slides to room temperature.

2. Prepare a labeled cDNA mixture by combining labeled nucleic acid and blocking agent(s), if desired (see Table 2), in a microcentrifuge tube. It may be necessary to first concentrate the mixture by ethanol precipitation or vacuum centrifugation. Suspend the probe mixture/pellet in an appropriate volume of ArrayHyb LowTemp Microarray Hybridization Buffer for the size cover slips being utilized (2.5 to 3 µl/cm^2 or 12.5 to 15 µl for 22 x 22 mm cover slips).

   Table 2.
   Blocking agents

Common Blocking Agents	Final Concentration (µg/ml)
Single-stranded DNA (D 8899)	100
Poly (dA) (P 0887)	400
Yeast tRNA (R 9001)	200
CoT-1 DNA®	400

3. Heat the ArrayHyb LowTemp/probe mixture at 60 °C for 5 minutes.

4. Centrifuge the contents to the bottom of the microcentrifuge tube. 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 °C 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. Post-Hybridization Wash

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

1. Fill separate wash dishes with Wash Buffers 1 (two dishes), 2, and 3.

2. Remove the slides one at a time from the humidity controlled incubator. Immerse the slides into the Wash Buffer 1 dish and gently remove the cover slips. Place the slides into a slide rack in the second Wash Buffer 1 dish. Incubate with gentle mixing on an orbital shaker for 5 minutes at room temperature.

3. Rinse the slides by plunging the slide rack into fresh distilled, deionized water dish several times. Remove excess liquid from rack and transfer to Wash Buffer 2. Incubate for 5 minutes at room temperature with gentle mixing on an orbital shaker.

4. Rinse the slides by plunging the slide rack into fresh distilled, deionized water dish several times. Remove excess liquid from rack and transfer to Wash Buffer 3. Incubate for 5 minutes at room temperature with gentle mixing on an orbital shaker.

5. After the third washing place the slide rack in a centrifuge equipped with a swinging bucket rotor for multiwell 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 at room temperature.

Slides are now ready for scanning.

Related Products

Product Name	Product Code
ArrayHyb™ Hybridization Buffer	A 7718
Arrayer Calibration Solution	C 2110
HybChecker™	H4411
GenElute™ PCR Purification Kit	GEN-PCR
Microarray Hybridization Wash Pack	M 2185
GenElute™ mRNA from Total RNA Kits	MRN-10 MRN-70
Poly $(dA)_n$	P 0887
Yeast tRNA	R 5636
GenElute™ Mammalian Total RNA Kits	RTN-10 RTN-70 RTN-350
SigmaScreen PLL Coated Slides for Microarrays	S 1313
SigmaScreen Ready-To-Coat Slides for Microarrays	S 2940
SigmaSpin™ Post-Reaction Clean-up Columns	S 5059
Hybridization Water Bath (115V or 220V)	Z36,765-6 Z36,766-4

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).
3. Schena, M. (Ed.), Microarray Biochip Technology, Eaton Publishing, Natick, MA, 2000, Product No. M 4309.
4. Schena, M. (Ed), DNA Microarrays, A Practical Approach, Oxford University Press, Oxford, England, 1999, Product Code D 6187.

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 is too high.	Decrease hybridization temperature (typically by 5 to 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 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 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 is too low.	Increase hybridization temperature (typically by 5 to 10 °C) to eliminate non-specific hybridization signal.
Irregular spot morphology	Poor printing of DNA	Dissolve the DNA in Standard Microarray Spotting Solution or another validated printing solution such as 3x SSC.
	Deformed spotting pins	Replace spotting pins.
Small spots	Printing solution too hydrophilic.	Alternate printing solutions could be tried. Standard Microarray Spotting Solution is recommended since it has been use tested with SigmaScreen APS Coated Slides for Microarrays.
	Slides too hydrophobic	Airborne environmental organics can adsorb to the slides making the glass surface more hydrophobic, thus driving the contact angle up. This is particularly problematic with arrayers that rely upon surface tension to transfer solution to the slide surface.
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.

BW/MAM 3/02