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ProductInformation

HybChecker™

Product Number **H 4411**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
HybChecker™ is an optimized hybridization solution containing fluorescent labeled random oligonucleotides to be used for quality control evaluation of printed microarray slides. The hybridization using fluorescent labeled random oligonucleotides will result in a fluorescent signal by binding of the oligonucleotides to the printed nucleic acids. This hybridization verifies the nucleic acid is present on the surface of the slide and available for hybridization. This permits assessment of spot morphology and detection of any spotting irregularities.

Precautions and Disclaimer
This product is for laboratory research use only. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The HybChecker solution is stable at –20 °C for at least 1 year. Avoid repeated freeze/thaw and exposure to light.

Procedure
I. DNA Printing
Print slides according to the arrayer manufacturer's protocol or a standard microarray printing protocol. Store printed slides desiccated at room temperature until ready for pre-treatment and hybridization. Always handle printed slides in a clean environment. Particles can lead to background signal during detection.

II. Post Print Treatment
Slides may be pre-treated as recommended for the slide type used. Typically this involves an SDS wash, boiling to denature double stranded DNA, a succinic anhydride blocking step (for amino slides), or sodium borohydride reduction (for aldehyde slides). Using HybChecker after pre-treatment will help to verify that the DNA samples are still present and available for hybridization.

III. Hybridization
HybChecker is provided as a ready to use solution.

1. Heat the required volume of HybChecker solution to 95 °C in a microcentrifuge tube in a heat block for approximately 1 minute.
2. Centrifuge the contents to the bottom of the microcentrifuge tube.
3. Carefully pipette the hybridization solution onto a cover slip.
   Note: The recommended volume of HybChecker for a glass coverslip (22 x 22 mm) is 20 μl. The appropriate volume required for larger coverslips or gasket type hybridization chambers should be determined by the end user.
4. 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.
5. Incubate the slides for 15 minutes to 1 hour at room temperature, protected from light.

IV. Post Hybridization Wash
Note: Do not allow the slides to dry out at any point during this procedure. This can cause non-specifically bound HybChecker to become permanently attached to the slide resulting in high backgrounds. Pre-made wash solutions are available as Microarray Hybridization Wash Pack (Product Code M 2185).

1. Remove the coverslip from each slide by immersing in Wash Buffer 1 (0.03% SDS, 1x SSC).
2. Wash the slides in Wash Buffer 1 for approximately 2 minutes at room temperature with mixing.
3. Repeat wash step using Wash Buffer 2 (0.2x SSC), followed by Wash Buffer 3 (0.05x SSC).
4. After the third washing, quickly dry the slides. A convenient way to dry slides is to transfer the slides to a slide rack (place paper towels below rack to absorb liquid) and spin at 50 to 100 x *g* for 5 to 10 minutes.
5. Immediately remove the slides from the centrifuge and store in a light-proof slide box.

6. Scan as soon as possible. The fluorescent dye, Atto 655, in HybChecker has an excitation wavelength of 655 nm and emission wavelength of 689 nm. It may be detected using either a Cy5™ or Bodipy™ 630-650 channel on commercially available fluorescent slide scanners such as the ScanArray 4000 (Packard).

Troubleshooting Guide

Problem	Cause	Solution
Spots appear smeared or as comets.	Recommended pre-treatment 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 may accomplished by a pre-treatment procedure that includes washing the slides in 0.5-1% SDS. 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.
	DNA not effectively immobilized to slide surface.	UV treatment required to effectively immobilize DNA to aminopropylsilane or poly-L-lysine slides. Use a reliable UV light source (such as BioLink BLX UV Crosslinker). Alternatively, print DNA on a slide surface that provides covalent linkage (aldehyde, etc.).
Irregular spot morphology	Deformed spotting pins	Replace spotting pins.
	Contamination in arrayed DNA	DNA must be purified properly prior to spotting. Dissolve the DNA in 3x SSC or another validated printing solution.
	Sub-optimal spotting solution used.	Alternative printing solutions could be tried. Sigma's Standard Microarray Spotting Solution (M 1435) has been tested with aminopropylsilane and poly-L-lysine slides.
Small spots	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 (split pin).
Low signal	Inadequate hybridization time period	Use a longer hybridization time.
	Probe has been exposed to light.	Protect labeled probes from exposure to light.
Background fluorescence	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:
SigmaScreen™ Coated Slides for Microarrays	S 9936
Poly-L-Lysine Coated Slides for Microarrays	S 1313
Arrayer Calibration Solution	C 2110
Standard Microarray Spotting Solution	M 1435
ArrayHyb™ Hybridization Buffer	A 7718
ArrayHyb LowTemp Hybridization Buffer	A 3095
Slide staining chambers/racks	S 6141
Microarray Hybridization Wash Pack	M 2185
GenElute™ PCR Purification Kit	NA-1020
BioLink BLX UV Crosslinker	Z37,537-3
GenElute™ Mammalian Total RNA Kits	RTN-10 RTN-70 RTN-350
GenElute™ mRNA from Total RNA Kits	MRN-10 MRN-70

References

1. Eisen, M.B., and Brown, P.O., DNA arrays for analysis of gene expression. Methods in Enzymology **303,** 179-205 (1999).
2. Schena, M., et al., Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science, **270,** 467-470 (1995).
3. Schena, M., et al., Parallel human genomic analysis: microarray-based expression monitoring of 1000 genes. Proc. Natl. Acad. Sci. USA, **93,** 10614-10619 (1996).
4. Schena, M., ed., *DNA Microarrays, A Practical Approach,* Oxford University Press (Oxford, England: 1999). Product Code D 6187.
5. Schena, M., ed., *Microarray Biochip Technology,* Eaton Publishing (Natick, MA: 2000). Product Code M 4309.

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