

SlideHyb™ Buffers

Glass Array Hybridization Buffers

Part Number AM8861-AM8863



A. Introduction

There are three unique SlideHyb Buffers developed for hybridization of DNA microarrays on either glass or nylon-modified slides. Because of the variability inherent in microarray manufacture and analysis, Ambion strongly recommends that users identify the optimal hybridization/wash temperature and SlideHyb Buffer any time a new microarray is used. Although it is not essential, we have found that it is useful to re-optimize even with a new lot of an identical microarray.

P/N	Product	Quantity	Storage
AM8861	SlideHyb™ Glass Array Hybridization Buffer #1	5 x 2 mL	4°C
AM8862	SlideHyb™ Glass Array Hybridization Buffer #2	5 x 2 mL	4°C
AM8863	SlideHyb™ Glass Array Hybridization Buffer #3	5 x 2 mL	4°C

B. Microarray Hybridization Protocol

1. Follow the manufacturer's recommendations for preparing and prewashing your microarray

2. Preheat SlideHyb Buffer to 68°C for 15–30 min

Swirl the SlideHyb Buffer until all precipitated material has dissolved.

The SlideHyb Buffers remain stable with repeated heating to 68°C, so the entire contents of the tubes can be pre-warmed to 68°C before removing an aliquot for hybridization.

3. Prepare labeled cDNA for hybridization

- a. Remove unincorporated nucleotides from the labeled cDNA using a size exclusion column (e.g. Ambion® NucAway™ columns (P/N AM10070), or Sephadex G-50.
- b. Precipitate, and resuspend the cDNA:
 - i. Add 0.1 volumes 3 M NaAcetate and 2.5 volumes 95–100% ethanol
 - ii. Incubate at -20°C for ≥ 20 min.
 - iii. Microfuge at top speed for 10–15 min at 4°C .
 - iv. Resuspend in 4 μL of 10 mM EDTA.
- c. Heat denature the labeled cDNA at 95°C for 10 min. Centrifuge to collect the liquid at the bottom of the tube.

4. Add warm SlideHyb Buffer to labeled cDNA

The volume of SlideHyb Buffer used will depend on the size of the microarray and the method used for hybridization. When coverslips are used, 40 μL per array will typically be appropriate. Other setups might require more SlideHyb Buffer.

5. Apply the SlideHyb/cDNA mixture to the microarray and hybridize overnight (14–24 hr)

Hybridize in a humidity chamber at 42 – 68°C (use the optimal temperature for your system if you have already determined it). Lower temperatures within this range will typically provide greater signal, but more cross-hybridization to non-target sequences.

6. Prepare low and high stringency wash buffers

Using 20X SSC and 10% SDS, prepare washing buffers. Ambion's recommendations follow:

Low stringency wash buffer

Final concentration	for 500 mL
2X SSC	50 mL of 20X SSC
0.5% SDS	25 mL of 10% SDS water to 500 mL

High stringency wash buffer

Final concentration	for 500 mL
0.5X SSC	12.5 mL of 20X SSC
0.5% SDS	25 mL of 10% SDS water to 500 mL

7. Preheat the wash buffers to the temperature used for hybridization
8. Wash the microarray 2 x 15 min in 50 mL of low stringency wash buffer at the temperature used for hybridization
9. Wash the microarray 2 x 15 min in 50 mL of high stringency wash buffer at the temperature used for hybridization
10. Follow the microarray manufacturer's instructions for hybridization signal detection

C. Troubleshooting Hybridization and Washing

1. High background

a. Incomplete solubilization of SlideHyb buffer and/or the labeled cDNA

These are the two primary causes of high background. Increase the amount of time used to preheat the SlideHyb, and be sure that there is no precipitate in it before adding it to the blot.

Vortexing the labeled cDNA vigorously during the heat denaturation step will help dissolve the pellet.

b. Inadequate removal of unincorporated labeled nucleotides.

It is crucial to remove unincorporated nucleotides from labeled cDNA to be used with microarrays. Ambion Nuc-Away™ spin columns are especially effective at removing nucleotides and small cDNA products from labeling reactions.

c. Inadequate reaction of primary amines during the post-processing of the microarray.

Using a fresh lot of anhydride is essential for removing unreacted amines from the array surface to avoid nonspecific interactions between the charged amines and labeled cDNAs.

2. Cross-hybridization

Stringency of the hybridization and washes is too low

- Increase hybridization or wash temperatures, or increase wash times to 30 min.
- Decrease the salt concentration in the high stringency wash to as low as 0.25X SSC.

3. Low signal

a. Problems with the labeled cDNA used in the hybridization

- Check that the RNA sample used to prepare the labeled cDNA is intact, and that the quantitation is accurate. Partially degraded RNA can not typically be used in this procedure.
- If the RNA appears to be intact, try making a fresh preparation of labeled cDNA and repeat the hybridization.

b. Hybridization and wash conditions are too stringent

- Lowering the hybridization temperature or the wash times and temperature can increase signal. Be aware that reducing stringency can lead to higher background and cross-hybridization.
- Omit the high stringency wash, or increase the salt concentration in the high stringency wash up to 1X SSC.

D. Quality Control

Functional testing

SlideHyb Buffer is functionally tested with an array membrane following the procedure described in this booklet.

Nuclease testing

Relevant kit components are tested in the following nuclease assays:

RNase activity

Meets or exceeds specification when a sample is incubated with labeled RNA and analyzed by PAGE.

Nonspecific endonuclease activity

Meets or exceeds specification when a sample is incubated with supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.

Exonuclease activity

Meets or exceeds specification when a sample is incubated with labeled double-stranded DNA, followed by PAGE analysis.

E. Safety Information

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely. Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining the MSDS

To obtain Material Safety Data Sheets (MSDSs) for any chemical product supplied by Applied Biosystems or Ambion:

- At **www.appliedbiosystems.com**, select **Support**, then **MSDS**. Search by chemical name, product name, product part number, or MSDS part number. Right-click to print or download the MSDS of interest.
- At **www.ambion.com**, go to the web catalog page for the product of interest. Click **MSDS**, then right-click to print or download.
- E-mail (MSDS_Inquiry_CCRM@appliedbiosystems.com) or telephone (650-554-2756; USA) your request, specifying the catalog or part number(s) and the name of the product(s). We will e-mail the associated MSDSs unless you request fax or postal delivery. Requests for postal delivery require 1–2 weeks for processing.

For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion, contact the chemical manufacturer.



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AB Applied Biosystems
Ambion®

U.S./Canada
(English) 800-327-3002
(French) 800-668-6913
Fax +1-512-651-0201

Europe
tel +44 (0)1480-373-020
fax +44 (0)1480-373-010

Japan
tel 81 (0) 3 5566 6230
fax 81 (0) 3 5566 6507

Direct free phone numbers, distributors:
www.appliedbiosystems.com