ULTRAhyb® Ultrasensitive Hybridization Buffer

Part Number AM8669, AM8670

Applied Biosystems

A. Product Description

ULTRAhyb® Hybridization Buffer maximizes the sensitivity of blot hybridizations by drastically increasing hybridization signal without increasing background. ULTRAhyb is unique in that it can be used either in sensitive mode: overnight hybridization, or in a fast mode: 2 hour hybridization. Using an overnight hybridization increases signal 20–50 fold over traditional hybridization buffers. Messages that require an overnight exposure to X-ray film using traditional hybridization buffers are generally easily detected using just a 2 hour hybridization in ULTRAhyb. Research by Ambion and others shows that only 1–5% of target molecules are bound to probe with an overnight incubation in standard hybridization buffers (Vernier et al. 1996 *Anal. Biochem.* **235**: 11–19, Brown, et al. unpublished). In contrast, 50–100% of target molecules are bound to probe in an overnight ULTRAhyb hybridization. ULTRAhyb can be used for assaying Northerns, Southerns, and dot/slot blots, using isotopic or nonisotopic RNA or DNA probes. Although some researchers have found that ULTRAhyb is compatible with array hybridization, we do not recommend it for this application. Instead, we suggest Ambion® SlideHyb™ Glass Array Hybridization Buffers (P/N AM8861, AM8862, AM8863).

IMPORTANT

Because ULTRAhyb will start to precipitate at temperatures below 25–30°C, it is typically not appropriate for use with oligonucleotide probes. We suggest ULTRAhyb-Oligo (P/N AM8663) for oligonucleotide probes.

Hybridization temperature and membrane compatibility

ULTRAhyb contains 50% formamide; it is compatible with positively charged, (such as Ambion BrightStar®-Plus) and neutral nylon membranes. ULTRAhyb can be used with nitrocellulose membranes, but only at a hybridization temperature of 68°C. Therefore, ULTRAhyb can be used with RNA probes and nitrocellulose blots, but ULTRAhyb should ***not*** be used with DNA probes and nitrocellulose blots.

Probe type	Immobilized nucleic acid	Hybridization temp
DNA	DNA	42°C
	RNA	42°C
RNA	DNA	42°C
	RNA	68°C

Wash solutions for use with ULTRAhyb

Typical wash solutions consisting of SSC or SSPE and SDS, including Ambion NorthernMax® Wash Buffers can be used with ULTRAhyb.

B. Components, Storage and Stability

P/N	Size	
AM8669	4 x 125 mL	ULTRAhyb
AM8670	125 mL	ULTRAhyb

For long term storage, ULTRAhyb should be stored at 4°C. If using the reagent frequently, store at room temperature where ULTRAhyb is stable for several months. The buffer remains stable with repeated heating to 68°C, thus the entire contents of the bottle can be prewarmed to 68°C before removing an appropriate amount for hybridization.

C. Hybridizing DNA Probes to RNA or DNA Blots

This procedure can also be used for hybridizing RNA probes to DNA blots, except that if nonisotopic RNA probes are used, increase the amount to 0.1 nM (~10 ng/mL of a 300 nt probe).

1. Preheat ULTRAhyb to 68°C. Swirl the buffer until all precipitated material has dissolved. ULTRAhyb is a complete prehybridization/hybridization buffer, it is not necessary to add any additional blocking agents.
2. Prehybridize the blot for 30 min at 42°C in enough ULTRAhyb to keep the membrane uniformly wet; this is ~6–10 mL depending on the size of the membrane and the size of the hybridization bottle or bag.
3. Double-stranded DNA probes must be denatured before hybridization. Add the following amount of probe to the prehybridized blot

Amount	Probe type
10^6 cpm/mL	radiolabeled probe
1–10 pM	nonisotopic probe*

* This is approximately 0.1 ng/mL of a 300 nt probe. Note that this is significantly less nonisotopic probe than the amount often suggested in blot hybridization procedures.
Up to 10 pM probe can be used for probes made by enzymatic incorporation of nonisotopically-modified nucleotide, whereas 1 pM should be used for probes made by chemical labeling methods such as the Ambion Psoralen-Biotin Kit.

4. Hybridize overnight (14–24 hr) at 42°C. The incubation time can be reduced to 2 hr for relatively abundant messages.
5. Discard the hybridization buffer and wash the blot 2 x 5 min in 2X SSC or SSPE, 0.1% SDS at 42°C.
   (Ambion NorthernMax® Low Stringency Wash Buffer #1, P/N AM8673 can be used.)
6. Wash the blot 2 x 15 min in 0.1X SSC or SSPE, 0.1% SDS at 42°C.
   (Ambion NorthernMax High Stringency Wash Buffer #2, P/N AM8674 can be used.)
7. Detect the probe.

D. Hybridizing RNA Probes to RNA Blots

1. Preheat ULTRAhyb to 68°C. Swirl the buffer until all precipitated material has dissolved. ULTRAhyb is a complete prehybridization/hybridization buffer, it is not necessary to add any additional blocking agents.
2. Prehybridize the blot for 30 min at 68°C in enough buffer to keep the membrane uniformly wet; this is ~6–10 mL depending on the size of the membrane and the size of the hybridization bottle or bag.
3. Add probe to the prehybridized blot

Amount	Probe type
10^6 cpm/mL	radiolabeled probe
0.1 nM	nonisotopic probe*

* approximately 10 ng/mL of a 300 nt probe.

4. Hybridize overnight (14–24 hr) at 68°C. The incubation time can be reduced to 2 hr for many messages.
5. Discard the hybridization buffer and wash the blot 2 x 5 min in 2X SSC or SSPE, 0.1% SDS at 68°C.
   (Ambion NorthernMax Low Stringency Wash, P/N AM8673 can be used.)
6. Wash the blot 2 x 15 min in 0.1X SSC or SSPE, 0.1% SDS at 68°C.
   (Ambion NorthernMax High Stringency Wash, P/N AM8674 can be used.)
7. Detect the probe.

E. Troubleshooting

ULTRAhyb is compatible with a range of probe types using the conditions suggested above. It might be necessary, however, to optimize hybridization and wash temperatures for your particular probe. Below is a list of common problems generally associated with membrane hybridizations.

High Background

Precipitates in the hybridization buffer

Inadequate solubilization of the hybridization buffer is one of the primary causes of high background. Increase the amount of time used to preheat the buffer, and be sure that there is no precipitate in the buffer before adding it to the blot. ULTRAhyb may start to precipitate at temperatures below 25–30°C.

Inadequate prehybridization

Increasing the blot prehybridization time from 30 min to 1 hr can decrease background.

Probe is too old

Isotopic probes that are several days old tend to produce higher background than freshly prepared probes. This is attributed to probe size; radiolytic decay reduces the size of the probe molecules over time.

Unincorporated radionucleotides

Although we don't ordinarily recommend removing free nucleotides, we have occasionally observed high background from unincorporated label. Remove free label by precipitation (0.5 M NH_4OAc and 2 volumes EtOH) or with a spin column designed for this purpose.

Inadequate hybridization stringency

If hybridizing at 42°C, try raising the hybridization temperature to 48°, or even to 55°C.

If hybridizing at 68°C, hybridization stringency is unlikely to be causing background. In our experience, raising the hybridization temperature above 68°C does not decrease background.

Ionic interactions

If the background signal makes the blot look uniformly dark, a high salt wash may help by minimizing ionic interactions between the probe and the hybridization membrane. To do this, after the ordinary washes, add a 2 x 15 min wash in 5X SSC or SSPE, 0.5% SDS at 68°C for RNA probes, or at 60°C for DNA probes.

Inadequate washing

Double check that your wash buffers contain SDS. Wash buffers lacking SDS are not recommended for use with ULTRAhyb.

Doubling the wash times and/or washing at higher temperatures can reduce background. Wash temperatures can be raised from 42°C to 55° or 60°C.

If you are washing at 68°C, inadequate washing is probably not causing your high background. In our experience, raising wash temperatures above 68°C does not decrease background.

Low Signal

Not enough probe, not enough label

Using less than the recommended amounts of probe, using low specific activity probe, or using less than full-length probes can lead to low signal. Each of these factors should be checked if low sensitivity is observed.

Hybridization and washes too stringent

Lower the hybridization temperature or the wash time and temperature. This may be especially helpful for oligonucleotide probes. Note that reducing stringency can lead to higher background and cross-hybridization.

Cross-Hybridization

The extreme sensitivity of ULTRAhyb may detect RNAs that are not the expected full-length target. Although much of the probe binding can be legitimate (hybridization to alternatively spliced, partially degraded, or closely related mRNAs), some might be hybridization to RNAs with only partial homology to the target.

Inadequate hybridization stringency

Increasing the hybridization and wash temperature 3–10°C can greatly reduce the levels of non-target hybridization. Simply reducing the amount of time used to expose the blot to film might also alleviate the problem.

Probes contain non-target sequence

The presence of vector sequence within the probe can cause hybridization to RNAs sharing sequence homology with the vector. If the probe template contains vector sequence, cleave it by restriction digestion and then gel purify the sequence of interest before labeling.

Too much nonisotopic probe

Nonisotopic probes can have problems with cross-hybridization, especially when they are used at 42°C. We have observed that lowering the probe concentration 10 to 100-fold in the hybridization reaction will greatly reduce nonspecific hybridization while having little if any impact on target-specific hybridization.

F. Solutions for Washing Blots

20X SSC

Concentration	Component
3 M	NaCl
0.3 M	sodium citrate, pH 7

20% (w/v) SDS

For 100 mL, dissolve 20 g Sodium Dodecyl Sulfate (SDS) in 80 mL of RNase-free water. Stir until the SDS is completely dissolved. Finally, bring the final volume to 100 mL with water.

SDS should not be inhaled, so use a fume hood or mask when weighing the powder.

2X SSC, 0.1% SDS

for 1 L	Component
100 mL	20X SSC
5 mL	20% SDS

0.1X SSC, 0.1% SDS

for 1 L	Component
5 mL	20X SSC
5 mL	20% SDS

G. Quality Control

Functional Testing

ULTRAhyb is tested functionally in a Northern blot using the Ambion NorthernMax® Kit.

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.

H. 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 goggles, 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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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