ULTRAhyb®-Oligo Buffer

Hybridization Buffer for Oligonucleotides
Part Number AM8663

Applied Biosystems

A. Product Description

ULTRAhyb®-Oligo Hybridization Buffer has been optimized specifically for hybridization of oligonucleotides in Northern analysis. ULTRAhyb-Oligo Hybridization Buffer maximizes the sensitivity of blot hybridizations by greatly increasing signal from end-labeled oligonucleotides without increasing the background.

Transfer membrane compatibility

ULTRAhyb-Oligo Buffer contains 25% formamide; it is compatible with positively charged nylon membranes (such as Ambion BrightStar®-Plus Membranes, P/N AM10100, AM10102, AM10104) and neutral nylon membranes.

Probe characteristics and hybridization temperature

DNA oligonucleotides with the characteristics listed below have been successfully hybridized overnight to RNA blots at 42°C.

Length	26–45 nucleotides
T_m value	78–90°C
GC content	47–62%
Specific activity	5 x 10^6 cpm/pmol

DNA oligonucleotides with a T_m outside of the range listed may require a different hybridization temperature that would have to be determined empirically.

Probe labeling and purification

A key to detecting rare messages with oligonucleotide probes is radiolabeling them to maximum specific activity. If you are using kinase to label the oligonucleotide probe, use a 2–5 fold molar excess of [γ-^{32}P]ATP relative to the oligonucleotide in the reaction. This will result in a high percentage of oligonucleotides bearing a radiolabel. Oligonucleotides should be labeled to at least 5 x 10^6 cpm/pmol, however for abundant messages, probe with a lower specific activity may suffice.

To minimize background, remove unincorporated [γ-^{32}P]ATP from labeled oligonucleotides before hybridization. This can be done using size exclusion chromatography (e.g., Ambion NucAway™ Spin Columns, P/N AM10070).

Wash solution for use with ULTRAhyb-Oligo Hybridization Buffer

A typical wash solution with 0.5% SDS and a buffer equivalent to 2X SSC or SSPE should be used for post-hybridization washes.

B. Storage and Stability

Long term storage

ULTRAhyb-Oligo Hybridization Buffer that will be used infrequently (less than approximately once a week) should be stored at 4°C.

NOTE

At 4°C, or at any temperature below 25–30°C, ULTRAhyb-Oligo Hybridization Buffer will start to precipitate. This is not a problem; it can be easily redissolved by heating to 68°C and swirling the bottle until any precipitated material is back in solution.

Storage for frequent usage

If you will be using ULTRAhyb-Oligo Hybridization Buffer every few days, it may be more convenient to store it at room temperature where, depending on the ambient temperature, it may not precipitate.

Stability

ULTRAhyb-Oligo Hybridization Buffer is guaranteed for 6 months from the date of shipment when stored at room temperature or 4°C.

C. Hybridization Instructions

IMPORTANT

ULTRAhyb-Oligo Hybridization Buffer is intended for hybridizing DNA oligonucleotides to RNA blots. It may not function well for other types of probes and blots.

1. Preheat ULTRAhyb-Oligo to 68°C

Swirl the bottle often to dissolve any precipitated material. If the ULTRAhyb-Oligo Hybridization Buffer has been stored at 4°C, precipitated material is expected and must be fully dissolved before using this product.

ULTRAhyb-Oligo Hybridization Buffer is a complete prehybridization/ hybridization buffer; therefore, it is not necessary to add additional blocking agents.

2. Prehybridize the blot for 30 min at 42°C

Use 1 mL ULTRAhyb-Oligo Hybridization Buffer per 10 cm^2 of membrane. Be certain that enough solution is present to keep the membrane uniformly wet.

3. Add 10^6 cpm/mL of the end labeled oligonucleotide

Hybridizing in roller bottles:

Stand the bottle on end, and add the labeled probe directly to the ULTRAhyb-Oligo solution used for the prehybridization.

Hybridizing in other containers:

Since it is important that undiluted probe solution does not touch the membrane, pour the ULTRAhyb-Oligo solution from the prehybridization into a 50 mL conical tube, add the labeled probe, mix by swirling, and then immediately pour the solution back into the container with the blot.

4. Hybridize overnight (14–24 hr) at 42°C

It may be possible to reduce the hybridization time for detection of relatively abundant messages, but the minimum necessary hybridization time would have to be determined empirically.

5. Wash the blot 2 x 30 min at 42°C

a. Following hybridization, discard the hybridization buffer appropriately.

b. Immediately pour at least 50 mL wash buffer consisting of 2X SSC or SSPE, 0.5% SDS onto the blot and incubate at 42°C for 30 min with gentle agitation.

c. Repeat with fresh wash buffer.

6. Expose the blot

Wrap the blot in plastic wrap and expose to film or to a phosphorimager screen. Suggested initial exposure times are listed below.

Message abundance	Initial exposure time
abundant	4 hours
moderate–rare	overnight to several days at –80°C with an intensifying screen

D. Troubleshooting

ULTRAhyb-Oligo Hybridization Buffer is compatible with a range of oligonucleotide probes varying in length, T_m value, GC content, and sequence. In some cases it may be necessary to optimize hybridization and wash temperatures for particular probes. 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. While warming the buffer at 68°C, thoroughly mix the buffer with a gentle swirling motion. Make certain there is no precipitate in the buffer before adding it to the blot. ULTRAhyb-Oligo Hybridization Buffer may start to precipitate at temperatures below 25–30°C.

Inadequate prehybridization

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

Unincorporated radionucleotides

We recommend that free label be removed with a spin column (i.e. Ambion NucAway™ Spin Columns P/N AM10070) before adding the oligonucleotide probe to the hybridization solution.

Inadequate washing

- Confirm that your wash buffer contains SDS. Wash buffers lacking SDS are not recommended for use with ULTRAhyb-Oligo Hybridization Buffer.
- Do the post hybridization washes in 5X SSC or SSPE, 0.5% SDS for 2 x 30 min. Increasing the salt concentration will help remove probe that is non-specifically bound to the membrane through electrostatic interactions.
- Double the post hybridization wash time in 2X SSC or SSPE, 0.5% SDS from 2 x 30 min to 4 x 30 min.
- If none of the above suggestions reduce non-specific background sufficiently, try increasing the posthybridization wash temperature by 5–10°C. This option is listed last because it may remove hybridized probe in addition to reducing background.

Low Signal

Not enough probe, low specific activity probe

The amount and specific activity of probe needed to obtain radioactive signal above background depends largely on the amount of target on the blot and the specific activity of the labeled probe. Maximize signal by using a molar excess of probe labeled to the highest possible specific activity.

Hybridization and/or washes are too stringent

If the oligonucleotide has a very low GC content or a low T_m, then lowering the hybridization temperature by 2–5°C may increase the signal by reducing the hybridization stringency. Note that this may increase background and cross-hybridization.

Alternatively washing can be done at room temperature instead of at 42°C.

Cross-Hybridization

The extreme sensitivity of ULTRAhyb-Oligo Hybridization Buffer may allow detection of RNAs that are not the expected full-length target. Although the probe binding could 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.

- The easiest way to reduce signal from cross hybridization (either cause) is to simply reduce the exposure time.

Inadequate hybridization stringency

Increasing the hybridization and wash temperatures can greatly reduce the levels of non-target hybridization.

- Increase the hybridization temperature by 2–5°C.
- Increase the wash temperature by 5–10°C.

Probe contains non-target sequence

If the oligonucleotide has sequence homology with other mRNAs, vectors, etc., cross-hybridization can result. If this is the case, redesign the probe to avoid sequence homology with targets other than the intended target.

E. Solutions for Washing Blots

1. 20X SSC

(This is also available as a premixed packet of powdered reagents: P/N AM9764)

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

2. 20% (w/v) SDS

(20% SDS is also available from Ambion: P/N AM9820) To make 100 mL 20% SDS, dissolve 20 g Sodium Dodecyl Sulfate (SDS) in 80 mL of RNase-free water. Stir until the SDS is completely dissolved, then bring the solution volume to 100 mL with water.

CAUTION

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

3. 2X SSC, 0.5% SDS

Amount	Component
875 mL	water
100 mL	20X SSC
25 mL	20% SDS

F. Safety Information

The MSDS for any chemical supplied by Applied Biosystems or Ambion is available to you free 24 hours a day.

IMPORTANT

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

To obtain Material Safety Data Sheets

- Material Safety Data Sheets (MSDSs) can be printed or downloaded from product-specific links on our website at the following address:
  www.ambion.com/techlib/msds
- Alternatively, e-mail your request to:
  MSDS_Inquiry_CCRM@appliedbiosystems.com. Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery.
- For customers without access to the internet or fax, our technical service department can fulfill MSDS requests placed by telephone or postal mail. (Requests for postal delivery require 1–2 weeks for processing.)

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.

G. Quality Control

Functional Testing

Detection of β-actin RNA in 0.2 µg of total RNA on a Northern blot using a labeled β-actin oligonucleotide.

Nuclease testing

Relevant kit components are tested in the following nuclease assays:

RNase activity

Meets or exceeds specification when a sample is incubated with 25 ng labeled RNA and analyzed by PAGE.

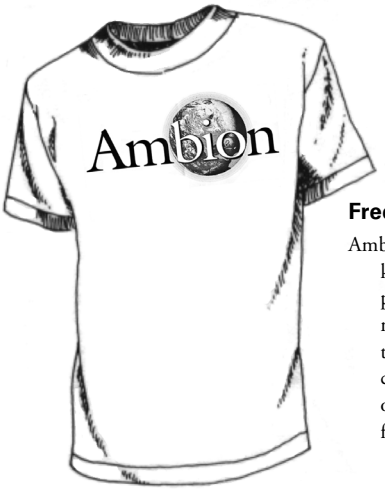
Nonspecific endonuclease activity

Meets or exceeds specification when a sample is incubated with 300 ng supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.

Exonuclease activity

Meets or exceeds specification when a sample is incubated with 40 ng labeled *Sau*3A fragments of pUC19, and analyzed by PAGE.

Send Us Your Reprint & We'll Give You The Shirt Off Our Back!



Free T-Shirt

Ambion appreciates when our kits and research systems are properly referenced in the materials and methods section of your research publication. To recognize this, we offer you an Ambion T-shirt for each publication.

To get your Ambion T-shirt, just send us:

1. the completed form below, and
2. a copy of your published paper that references an Ambion product

Name	Name (if different)
Institution	Size: M L XL
Address	Address to send shirt to (if different)
City State Zip	City State Zip

Mail to: Ambion, Inc.
2130 Woodward St.
Austin, TX 78744

Offer good while supplies last. Shirts and sizes are subject to availability. Ambion reserves the

Manual 8663M Revision B **Revision Date: April 22, 2008**

For research use only. Not for use in diagnostic procedures.

Information in this document is subject to change without notice. Applied Biosystems assumes no responsibility for any errors that may appear in this document.

Applied Biosystems disclaims all warranties with respect to this document, expressed or implied, including but not limited to those of merchantability or fitness for a particular purpose. In no event shall Applied Biosystems be liable, whether in contract, tort, warranty, or under any statute or on any other basis for special, incidental, indirect, punitive, multiple or consequential damages in connection with or arising from this document, including but not limited to the use thereof.

Literature Citation: When you are describing a procedure utilizing this product in a Materials and Methods Section for publication, we would appreciate that you refer to it as the Manual Title™ Kit.

Warranty and Liability: Applied Biosystems is committed to delivering superior product quality and performance, supported by industry-leading global service and technical support teams. Warranty information for the accompanying consumable product is available at www.ambion.com/info/warranty in "Limited Warranty for Consumables," which is subject to the exclusions, conditions, exceptions, and limitations set forth under the caption "EXCLUSIONS, CONDITIONS, EXCEPTIONS, AND LIMITATIONS" in the full warranty statement. Please contact Applied Biosystems if you have any questions about our warranties or would like information about post-warranty support.

Trademarks: Applied Biosystems, AB (Design), Ambion, BrightStar and ULTRAhyb are registered trademarks; and NucAway is a trademark of Applera Corporation or its subsidiaries in the US and/or certain other countries. All other trademarks are the sole property of their respective owners.

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