

ULTRAhyb™ Ultrasensitive Hybridization Buffer

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Product description

The Ambion™ ULTRAhyb™ Ultrasensitive Hybridization Buffer (ULTRAhyb™) is used for Northern, Southern, and dot/slot blot hybridizations.

Key features of the kit include:

- Can be used in sensitive mode (overnight hybridization) or fast mode (2 hour hybridization).
- As few as 10,000 molecules can be detected.
- Compatible with RNA and DNA probes labeled isotopically and nonisotopically.

ULTRAhyb™ contains 50% formamide and is compatible with positively charged membranes. It contains a unique blend of hybridization accelerators and blocking agents that greatly enhance the levels of hybridization.

Note: Because ULTRAhyb™ starts to precipitate at temperatures below 25–30°C, it is typically not appropriate for use with oligonucleotide probes. We suggest the ULTRAhyb™-Oligo Buffer (Cat. No. AM8663) for oligonucleotide probes.

Hybridization temperature and membrane compatibility

ULTRAhyb™ contains 50% formamide; it is compatible with positively charged and neutral nylon membranes (such as BrightStar™-Plus Positively Charged Nylon Membrane, Cat. No. MAN10102). 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 temperature
DNA	DNA	42°C
	RNA	42°C
RNA	DNA	42°C
	RNA	68°C

Compatible wash solutions

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

Contents and storage

Contents	Cat. No. AM8669	Cat. No. AM8670	Storage
ULTRAhyb™ Ultrasensitive Hybridization Buffer	4 x 125 mL	125 mL	<ul style="list-style-type: none">• 4°C^[1]• Room temperature^[2]

^[1] Longer than several months

^[2] Several months or less.

ULTRAhyb™ Ultrasensitive Hybridization Buffer appearance upon its arrival and during the storage can vary from white, pink or yellow. The variation in buffer color does not affect product performance or stability. The buffer precipitates at 4°C, or at any temperature below 25–30°C. This does not affect product performance. The precipitate should be redissolved by heating to 68°C and swirling the bottle until any precipitated material is back in solution. 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.

Hybridize 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 ^[1]

^[1] *This is approximately 0.1 ng/mL of a 300 nt probe. 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.

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, Cat. No. 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, Cat. No. AM8674, can be used.)
7. Detect the probe.

Hybridize 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 minutes 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 ^[1]

^[1] Approximately 10 ng/mL of a 300 nucleotide probe.

4. Hybridize overnight (14–24 hours) at 68°C. The incubation time can be reduced to 2 hours for many messages.

5. Discard the hybridization buffer and wash the blot 2 x 5 minutes in 2X SSC or SSPE, 0.1% SDS at 68°C. (NorthernMax™ Low Stringency Wash Buffer #1, Cat. No. AM8673 can be used.)
6. Wash the blot 2 x 15 minutes in 0.1X SSC or SSPE, 0.1% SDS at 68°C. (NorthernMax™ High Stringency Wash Buffer #2, Cat. No. AM8674 can be used.)
7. Detect the probe.

Solutions

Solution	Instructions
20X SSC (3 M NaCl, 0.3 M sodium citrate pH 7)	AM9763
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.  CAUTION! SDS should not be inhaled use a fume hood or mask when weighing the powder.
2X SSC, 0.1% SDS	Per liter: <ul style="list-style-type: none"> • 100 mL 20X SSC • 5 mL 20% SDS
0.1X SSC, 0.1% SDS	Per liter: <ul style="list-style-type: none"> • 5 mL 20X SSC • 5 mL 20% SDS

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.

Safety



WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the “Documentation and Support” section in this document.



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.



WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.



WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety



WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at: <https://www.cdc.gov/labs/pdf/CDC-Biosafety%20microbiological%20Biomedical%20Laboratories-2009-P.pdf>
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at: www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Troubleshooting and FAQs

Visit our online Support Centers and FAQ database for tips and tricks for conducting your experiment, troubleshooting information, and FAQs. The online FAQ database is frequently updated to ensure accurate and thorough content.

- For the Nucleic acid purification and analysis Support Center: <http://thermofisher.com/napsupport>
- For FAQs for this product: <http://thermofisher.com/AM8669faqs>
- To browse the FAQ database and search using keywords: thermofisher.com/faqs

Documentation and support

Customer and technical support

Visit [thermofisher.com/support](https://www.thermofisher.com/support) for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
 - Product FAQs
 - Software, patches, and updates
 - Training for many applications and instruments
- Order and web support
- Product documentation
 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.



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For descriptions of symbols on product labels or product documents, go to [thermofisher.com/symbols-definition](https://www.thermofisher.com/symbols-definition).

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Revision	Date	Description
A.0	22 December 2020	<ul style="list-style-type: none">• Baseline for revision. Updated from Doc. Part No. 8670M Revision B.• Product description aligned to content on thermofisher.com.• Quality Control section updated.

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