ULTRAhyb™–Oligo Buffer

Hybridization buffer for oligonucleotides

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Product description

Ambion™ ULTRAhyb™–Oligo Buffer is specifically formulated for use with oligonucleotide probes in Northern analysis. It contains a unique blend of hybridization accelerators and blocking agents that greatly enhance the levels of hybridization so that signals that once took days to visualize are now apparent in hours.

Key features of the kit include:

- Increased sensitivity of northern analysis.
- Less probe used per blot.
- Decreased hybridization time (2 hours).

IMPORTANT! ULTRAhyb™–Oligo Buffer is intended for hybridizing DNA oligonucleotides to RNA blots. It may not function well for other types of probes and blots.

Transfer membrane compatibility

ULTRAhyb™–Oligo Buffer contains 25% formamide; it is compatible with positively charged nylon membranes (such as Ambion™ BrightStar ™–Plus Positively Charged Nylon Membrane; Cat. Nos. 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.
- DNA oligonucleotides with a T_m outside of the range listed may require a different hybridization temperature that would have to be determined empirically.

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

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 results 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 (for example, Ambion™ NucAway™ Spin Columns, Cat. No. AM10070).

Wash solution for use with ULTRAhyb™–Oligo 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.

Contents and storage

Contents	Amount	Storage
ULTRAhyb™–Oligo Buffer	125 mL	• 4°C[1] • Room temperature[2]

[1] Longer than several months
[2] Several months or less.

ULTRAhyb™–Oligo 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 probes

1. Preheat ULTRAhyb™–Oligo Buffer to 68°C.
2. If there is precipitate in ULTRAhyb™–Oligo Buffer, swirl the bottle until the precipitate is completely dissolved.
3. Prehybridize the blot for 30 minutes at 42°C. Use 1 mL ULTRAhyb™–Oligo Buffer per 10 cm^2 of membrane.

   Be certain that enough solution is present to keep the membrane uniformly wet.
4. 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 Buffer 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 Buffer 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.
5. 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.
6. Discard the hybridization buffer.
7. Pour at least 50 mL wash buffer consisting of 2X SSC or SSPE, 0.5% SDS onto the blot, then incubate at 42°C for 30 minutes with gentle agitation.

8. Discard the wash buffer.

9. Repeat steps step 7–step 8.

10. 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.

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, 0.5% SDS	Per liter: • 875 mL nuclease-free water • 100 mL 20X SSC (3 M NaCl, 0.3 M sodium citrate pH 7) • 25 mL 20% SDS

Quality Control

Functional testing

Target RNA detection in total RNA on a Northern-blot using a labeled 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 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.

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/AM8663faqs**
- To browse the FAQ database and search using keywords: **thermofisher.com/faqs**

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.

Chemical safety

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-BiosafetymicrobiologicalBiomedicalLaboratories-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

Documentation and support

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  - 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.

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Revision	Date	Description
A.0	22 December 2020	• Baseline for revision. Updated from Doc. Part No. 8663M Revision B. • Product description aligned to content on thermofisher.com. • Quality Control section updated.

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