

# BrightStar<sup>®</sup> Psoralen-Biotin Kit

(Part Number AM1480)

## *Protocol*

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# I. Introduction

## A. Overview

Psoralens are planar tricyclic compounds which have a natural affinity for nucleic acids and intercalate between bases in a manner similar to ethidium bromide. Upon irradiation with long wave UV light, psoralens become covalently attached to the nucleic acid, preferentially to thymidines, and also to uridines and cytidines. Ambion® BrightStar® Psoralen-Biotin Nonisotopic Labeling Kit uses the intercalating property of psoralens to efficiently label nucleic acids with biotin, using a BrightStar Psoralen-Biotin conjugate. Intercalation does not compromise hybridization efficiency when the labeled nucleic acid is used as a probe. Ambion BrightStar Psoralen-Biotin Labeling Kit is optimized to produce high specific activity biotinylated RNA and DNA probes. Labeling reactions are simple and fast with predictable yields, and the procedure is entirely scalable, permitting large amounts of material to be labeled at once. The labeling is carried out by mixing the nucleic acid with the BrightStar Psoralen-Biotin in a 96-well plate and exposing to long wavelength (365 nm) UV light. The BrightStar Psoralen-Biotin becomes covalently linked to the nucleic acid in 15–60 min. Any excess BrightStar Psoralen-Biotin is removed by butanol extraction and the probe is ready for use. Probes are stable for at least a year when stored at  $-80^{\circ}\text{C}$ . Nucleic acid solutions in the pH range of 2.5 to 10 can be used. The total salt concentration of the solution must be less than 20 mM.



### CAUTION

*UV lamps produce radiation which can be hazardous to eyes and skin. Therefore, use protective goggles or face shield, and appropriate clothing when doing BrightStar Psoralen-Biotin reactions.*



### NOTE

*The BrightStar Psoralen-Biotin is light sensitive and must be stored in the dark at  $-20^{\circ}\text{C}$ .*

## B. Reagents Provided with the Kit and Storage

The kit contains reagents for 20 labeling reactions or for labeling a total of 10 µg of nucleic acid.

Amount	Component	Storage
4.17 µg	BrightStar Psoralen-Biotin	-20°C (dark)
400 µL	Dimethylformamide (DMF)	-20°C
2 mL	TE Buffer 10 mM Tris-HCl pH 8.0 1 mM EDTA	-20°C
15 µL	Unlabeled Control DNA 0.1 µg/µL in TE Buffer	-20°C
20 µL	Labeled Control DNA 1 ng/µL in TE Buffer	-20°C
13 mL	Water-saturated 1-Butanol Shake well before <i>each</i> use	-20°C

The Labeled and Unlabeled Control DNAs are a linear 4 kb plasmid containing a 1 kb insert of the mouse Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) gene.

## C. Materials not Provided with the Kit

### Long wave UV lamp

A long wave UV is used to link the biotin moiety of the BrightStar Psoralen-Biotin to a nucleic acid. It is essential that a *long wavelength* UV lamp (365 nm) be used (e.g. P/N AM12750).

### 96-well plates

96-well plates are very convenient vessels for BrightStar Psoralen-Biotin labeling because they simultaneously provide a support for the UV light source and a raft in the ice bucket for the material being labeled. Other vessels that allow the UV light source to be less than 2 cm from the sample nucleic acid can be used, just make sure that water from melting ice does not get into the samples during the 45 min incubation.

The 96-well plates used for BrightStar Psoralen-Biotin labeling, should be “untreated”. Any coating such as those used on some tissue culture plates will absorb the nucleic acid sample, severely decreasing yield. In addition, when labeling RNA, the plates used must be RNase-free. This can be accomplished by treating the plate/wells with Ambion RNaseZap® Solution, or by soaking for 30 min in 0.1 N NaOH, rinsing in DEPC-treated water and drying.

### (optional) Diethyl ether

Molecular biology grade diethyl ether can be used to remove trace amounts of 1-Butanol from BrightStar Psoralen-Biotin reaction products if desired.

## D. Related Products Available from Applied Biosystems

Portable UV Light P/N AM12750	A low cost, pocket-sized, long wavelength UV light. This light is ideal for catalyzing BrightStar® Psoralen-Biotin labeling reactions
BrightStar® BioDetect™ P/N AM1930	Low background, high sensitivity detection kit for biotinylated RNA and DNA probes. This nonisotopic detection system is compatible with Northern, Southern, dot blot and nuclease protection assays.
BrightStar®-Plus Membrane P/N AM10100-AM10104	Positively-charged nylon membranes recommended for use with Ambion BrightStar® nonisotopic labeling and detection products. These membranes are an excellent choice for Northern and other blot hybridizations.
DNA-free™ Reagents P/N AM1906	DNase treatment and removal reagents. This product contains Ambion's ultra-high quality RNase-free DNase I and reaction buffer for degrading DNA. It is ideal for removing contaminating DNA from RNA preparations. A novel reagent for removing the DNase without the hassles or hazards of phenol extraction or alcohol precipitation is also included.
MEGAscript® Kits P/N AM1330-AM1338	High yield transcription kits for production of large amounts of RNA. By employing Ambion's novel, patented MEGAscript® technology, these kits use concentrations of nucleotides that would normally inhibit the RNA polymerases, resulting in ultra high-yield transcription reactions. Kits are available with T7, SP6, and/or T3 RNA polymerase.
MAXIscript® Kits P/N AM1308-AM1326	MAXIscript Kits are designed for synthesis of high specific-activity RNA probes with specific activities reaching $1 \times 10^9$ cpm/μg in just 10 minutes. MAXIscript Kits are available for DNA templates containing T7, T3, and SP6 promoters.

## II. Brief Procedure for Experienced Users

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The following procedure gives instructions to label 5–500 ng of nucleic acid in a 10  $\mu$ L reaction. The reaction can be scaled up proportionally if desired. For volumes  $\geq 100$   $\mu$ L, divide the sample into 2 or more wells.

**IMPORTANT**

Steps 3–5 must be done in reduced light (e.g. in a dim room).

1. Place a clean untreated 96-well plate on an ice bath.
2. Denature the sample by heating to 100°C for 10 min, and then rapidly cooling the mixture in a dry ice/alcohol bath. The sample will freeze.

3. Centrifuge the vial containing the lyophilized BrightStar Psoralen-Biotin for ~15 seconds at 7,000 x g. Reconstitute the BrightStar Psoralen-Biotin in 33  $\mu$ L DMF. Pipet up and down a few times to get the BrightStar Psoralen-Biotin into solution. The solution should be stored at 4°C, in the dark.

4. Thaw the frozen sample by rolling it between gloved hands, and immediately add 1  $\mu$ L of the BrightStar Psoralen-Biotin to 10  $\mu$ L of the nucleic acid solution in a microfuge tube. Mix and transfer the sample to a well in the 96-well plate (on ice). Alternatively, add BrightStar Psoralen-Biotin to nucleic acid already in the 96-well plate and mix with a pipet tip.

The nucleic acid solution should have a final concentration of 0.5–50 ng/ $\mu$ L. The pH of the nucleic acid solution should be between 2.5 and 10, and the salt concentration should be less than 20 mM.

**IMPORTANT**

Use **only** long wavelength (365 nm) UV lamps. Medium wavelength (312 nm) and short wavelength (254 nm) lamps **cannot** be used. The distance between the lamp source and the sample should be 2 cm or less.

5. Place a 365 nm UV light source on the plate directly over the sample and irradiate the sample for 45 min.
6. Dilute the sample to 100  $\mu$ L by adding 89  $\mu$ L of TE Buffer and transfer the mixture to a clean microfuge tube.
7. Add 200  $\mu$ L of Water-saturated 1-Butanol (shake well before use). Vortex well and microcentrifuge for 1 min at 7,000 x g. Pipet off the top 1-Butanol layer and repeat.
8. (optional) The traces of 1-Butanol may be removed by extracting with 2 volumes of water-saturated diethyl ether, microcentrifuging and pipetting off the ether.
9. Store the biotin-labeled nucleic acid at –20°C for short term storage (up to a few weeks) or at –80°C for long term storage (months to a year).

### III. Detailed Procedure for New Users

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#### A. Important Information for All Users

Please read all sections of this protocol before proceeding with any experiments.

- Nucleic acids cannot be quantitated by absorbance at 260 nm after BrightStar Psoralen-Biotin labeling because Psoralen absorbs at this wavelength, interfering with an accurate reading, and potentially destroying the crosslinks that connect biotin to the nucleic acid. It is recommended that quantitation is done before labeling with BrightStar Psoralen-Biotin. Yield from the procedure will be 90–100%, with a slight loss resulting from the butanol extraction.
- BrightStar Psoralen-Biotin interacts preferentially with T residues in DNA and U residues in RNA. It also interacts with C residues. Because of this, the efficiency of labeling small (<100 base) polynucleotides varies with the proportion of U, T and C residues, and may be affected by their context.
- Use only nuclease-free water for rinsing glassware. If necessary, use a nuclease decontamination reagent (such as RNaseZap® Solution) for cleaning glassware before rinsing with water.
- Try the labeling reaction using the Unlabeled Control DNA at least once. This will help you to interpret your results and will also ensure that the reagents are performing according to specifications.
- Thin-walled PCR tubes are ideal for heat denaturing and quick-chilling nucleic acids before labeling. **Very rapid chilling** of nucleic acids after heat denaturation is essential to prevent them from re-annealing. This can be achieved by placing the tubes in powdered dry ice/methanol, in liquid nitrogen, or in ice water.

#### B. Sample Purity and Concentration

Almost any nucleic acid can be labeled with this kit (e.g. PCR product, cDNA, linearized plasmid, in vitro transcript etc.). Oligonucleotides as small as 47-mers have been successfully labeled using BrightStar Psoralen-Biotin. The procedure below gives instructions to label 5–500 ng of sample in a 10 µL reaction (5 ng is not enough labeled nucleic acid for most applications). The reaction can be scaled up proportionally to accommodate more nucleic acid if desired. We have labeled up to 100 µL of sample in a single well of a 96-well plate without observing any negative effect on labeling efficiency. For volumes over 100 µL we recommend dividing the sample into 2 or more wells in the 96-well plate for the labeling reaction.

**1. Sample purity****There should be no free nucleotides in the preparation (e.g. from PCR)**

If the template is a PCR product, remove the free nucleotides with a spin column or with 2 successive isopropanol precipitations (see section [4](#) on page 13) before attempting to label it using BrightStar Psoralen-Biotin.

**RNA samples must be free of DNA e.g. transcription template**

To remove DNA, treat the RNA with DNase, followed by Proteinase K/SDS digestion and acid phenol extraction (procedures [1-3](#) on page 13); finally, precipitate the RNA with ammonium acetate and ethanol. Alternatively, you can use Ambion DNA-free™ DNase Treatment and Removal Kit to simplify this process substantially, (see section [1.D](#) on page 3).

**Samples must be free of proteins**

Remove proteins with a Proteinase K/SDS digestion followed by phenol/chloroform extraction and precipitation (procedures [2-4](#) on page 13).

**Nucleic acid solutions should be free of phenol, or alcohol contaminants, the pH should be between 2.5 and 10, and the salt concentration should be less than 20 mM.**

If the sample does not meet these requirements, precipitate it (see procedure [4](#) on page 13), and resuspend it in TE Buffer to 50 ng/μL.

**2. Sample concentration****Sample concentration should be 0.5–50 ng/μL**

If your sample is a precipitated nucleic acid, dissolve it in TE Buffer to a concentration of 50 ng/μL.

If the solution is too concentrated, dilute it with the TE Buffer provided. If it is too dilute, concentrate it by precipitation (see procedure [4](#) on page 13).

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**C. Nucleic Acid Labeling****1. Set up a 96-well plate in an ice bath**

Place a clean untreated (non-coated) 96-well plate on an ice bath. If necessary wash the 96-well plate with RNaseZap to remove any nucleases, rinse with dH<sub>2</sub>O and air dry.

**2. Denature sample at 100°C for 10 min**

We recommend using thin-wall (PCR) tubes for heat denaturation and cooling, because the tube contents can be cooled more quickly than in ordinary microfuge tubes.

Heat 10 μL (5–500 ng) of the sample in a microcentrifuge tube for at least 10 min in a boiling water bath or heat block at 100°C. Samples that are not in TE should be in a solution with at least 1 mM EDTA so that they don't degrade during the heat denaturation

### 3. Immediately quick-chill the sample by placing in a dry ice/alcohol bath

If a large volume of nucleic acid solution is being heated, it may be necessary to set the heat block to about 110°C to ensure complete denaturation.

Extremely rapid cooling is critical for efficient labeling.

Liquid nitrogen, or an ice-water bath may be used instead of dry ice/alcohol.

The sample will freeze, simply leave it frozen until you are ready to add the BrightStar Psoralen-Biotin in step 5.

If you use an ice-water bath, it must be a slush and not simply ice alone.



#### IMPORTANT

*Steps 4–6 must be done in reduced light. A dim room will suffice. If desired, the lights can be completely turned off after the reaction is set up.*

### 4. First time use of kit: dissolve the BrightStar Psoralen-Biotin in 33 µL Dimethylformamide

Centrifuge the vial containing the lyophilized BrightStar Psoralen-Biotin for ~15 seconds at 7,000 x g.

In dim light, reconstitute the BrightStar Psoralen-Biotin in 33 µL of DMF that is provided with the kit. Pipet up and down a few times to get the BrightStar Psoralen-Biotin into solution. The solution should be stored at 4°C, in the dark.

### 5. Add 1 µL BrightStar Psoralen-Biotin to 10 µL nucleic acid sample

Thaw the sample by rolling it between gloved hands. As soon as it is thawed, add 1 µL BrightStar Psoralen-Biotin, and mix well.

Transfer the reaction to a well in the 96-well plate in ice (from step 1). Alternatively, the BrightStar Psoralen-Biotin can be added to the nucleic acid already in a well of the 96-well plate. Mix the solution with a pipet tip.

If the sample volume is >10 µL, use proportionally more BrightStar Psoralen-Biotin. For example, a 50 µL sample would require 5 µL BrightStar Psoralen-Biotin.

### 6. Irradiate at 365 nm for 45 min

Place a 365 nm UV light source on the plate directly over the sample and irradiate for 45 min. (The dual wavelength hand held UV lamps found in many labs are suitable. Make sure the lamp is set to 365 nm and *not* to 254 nm). We have not encountered any degradation or crosslinking of nucleic acids from this UV treatment.



#### IMPORTANT

*Medium wavelength (312 nm) and short wavelength (254 nm) lamps should not be used. Also, be sure that nothing is between the sample and the light source. The irradiation will be compromised if there is plastic wrap or glass between the samples and the UV light. By the same token, samples cannot be irradiated through the bottom of the 96-well plate. e.g., on a transilluminator. The distance between the lamp source and the sample should be ≤2 cm.*

The biotinylation is now complete, so for subsequent steps it is not necessary to shield samples from ambient light.

**7. Dilute the sample to 100  $\mu$ L by adding 89  $\mu$ L TE Buffer**

This is done in order to avoid any sample loss during subsequent extraction with 1-Butanol. Transfer the sample from the 96-well plate to a clean microfuge tube.

**8. Extract twice with 200  $\mu$ L Water Saturated 1-Butanol**

Remove non-crosslinked BrightStar Psoralen-Biotin by butanol extraction as follows:

- a. Add 200  $\mu$ L of Water-saturated 1-Butanol (shake the butanol well before use, and use the top layer).
- b. Vortex well and microcentrifuge for 1 min at 7,000  $\times$  g.
- c. The butanol layer will be on top, pipet it off, and do a second extraction. Remove as much of the 1-Butanol as possible.

The traces of 1-Butanol that may remain (noticed as a cloudiness when the sample is stored in a refrigerator) will not interfere with future uses of the labeled probe.

The probe is now ready for use.

**9. (optional) Extract with diethyl ether**

Trace amounts of 1-Butanol can be removed by extracting with 2 volumes of water-saturated diethyl ether and pipetting off the diethyl ether.

**10. Storage of biotinylated nucleic acids**

Store biotinylated nucleic acids at  $-20^{\circ}\text{C}$  for short term storage (up to a few weeks) and at  $-80^{\circ}\text{C}$  for long term storage (months to a year).

To avoid repeated freeze-thawing, large amounts of labeled probe should be stored in the dark in small aliquots at  $-80^{\circ}\text{C}$ . The currently used aliquot may be stored at  $-20^{\circ}\text{C}$ . The labeled nucleic acid should be stable for at least one year at  $-80^{\circ}\text{C}$  in the absence of nuclease contamination.

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## D. Using BrightStar Psoralen-Biotin Labeled Probes

### 1. Concentration for use in blot hybridization

#### Standard Hybridization Buffers

Use 0.1 nM final concentration of biotinylated probe in standard Northern, Southern, dot blot or colony hybridization buffer.

#### ULTRAhyb<sup>®</sup> Buffer

The probe concentration that should be used in Ambion ULTRAhyb<sup>®</sup> buffer depends on the probe type.

RNA probes: 0.1 nM (~10 ng/mL of a 300 nt probe)

DNA probes: 1 pM (~0.1 ng/mL of a 300 nt probe)

### 2. Heat denaturation of DNA probes

dsDNA probes must be denatured by heat treatment before use in hybridization applications. It is advisable to re-denature probes with each use.

a. Dilute the probe ~10-fold with 10 mM EDTA (use a minimum of 50  $\mu$ L).

b. Incubate the diluted probe at 90°C for 10 min.

c. Add the denatured probe directly to the hybridization buffer and quickly mix thoroughly.

Alternatively the probe can be quick chilled in ice water or in dry ice/alcohol after heat denaturation and stored in ice until it is added to the hybridization buffer.

### 3. Storage of hybridized membranes

If necessary, membranes can be stored damp at -20°C for several weeks without affecting probe detection. Membranes that have inadvertently been allowed to dry can still be subjected to biotin detection, but background may be higher.

## IV. Troubleshooting

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### A. Do the Positive Control

To ensure the kit is performing well, Ambion provides Unlabeled and Labeled Control DNAs. The control reaction consists of using the BrightStar Psoralen-Biotin Kit to biotinylate the Unlabeled Control DNA. Then both Control DNAs (labeled-by-Ambion and labeled-by-user) are subjected to a biotin detection procedure, and the results are compared.

#### 1. BrightStar Psoralen-Biotin label the Unlabeled Control DNA

The Unlabeled Control DNA is supplied at 100 ng/μL in TE Buffer; dilute 5 μL of this control DNA with 5 μL TE Buffer for a final concentration of 50 ng/μL. Then label 10 μL of the diluted DNA following the procedure described in section [III.C. Nucleic Acid Labeling](#) starting on page [6](#).

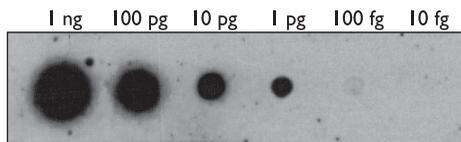
#### 2. Detect the biotin

To assess the outcome of the positive control reaction and/or any biotinylation reactions with experimental nucleic acids, compare the detection of the biotin on the Labeled Control DNA provided with the kit, with the sample(s) labeled in your lab. When doing this comparison, remember that the amount of biotin incorporated will be directly proportional to the number of T, U, or C residues in the nucleic acid. This will mostly be a function of length (the Control DNAs are 4 kb – so an equivalent signal from a 1 kb nucleic acid would be 25% of the signal from the Control DNA).

- a. Make 10-fold dilutions of biotinylated samples from 1 ng/μL down to 1 fg/μL.
- b. Carefully spot 1 μL aliquots onto positively charged nylon membrane. (We usually put the membrane on a piece of plastic wrap or on a clean paper towel while applying the spots.)
- c. Crosslink the nucleic acids to the membrane using UV light or by baking at 80°C for 15 min.
- d. Detect the biotin using your biotin detection system of choice (e.g. Ambion's BrightStar BioDetect Nonisotopic Detection Kit).

**Expected result:**

The DNAs i.e. labeled-by-Ambion and labeled-by-the-user, should be easily detectable at 1 pg.



**Figure 1. Limit of Sensitivity for Detection of a 1 kb Biotinylated DNA Probe**

A dilution series of a 1 kb biotinylated DNA probe was spotted onto an Ambion BrightStar-Plus membrane, and detected using the BrightStar BioDetect Kit. A 45 min exposure to X-ray film revealed a sensitivity limit of ~100 fg.

**B. Inefficient Labeling of the Nucleic Acid**

If the positive controls provided with the kit are detectable at 100 fg to 1 pg, indicating that the BrightStar Psoralen-Biotin Kit and the biotin detection system are both performing as expected, but the test nucleic acid appears not to be labeling efficiently, we recommend running the material on a gel to see if it is intact. If the test nucleic acid is intact as determined by gel electrophoresis, then there may be inhibitors present which are interfering with the labeling reaction. The presence of free nucleotides from an in vitro transcription reaction or a PCR could cause this problem. Free nucleotides can be removed by precipitation with salt/ethanol or isopropanol, followed by a 70% ethanol wash.

There are a number of factors that are important for the efficient labeling of nucleic acids using BrightStar Psoralen-Biotin. It is imperative that the labeling procedure be done as specified. Some of the crucial variables are listed below:

1. Make sure that the correct wavelength of UV light is used. Only long wave UV (365 nm) works. Shortwave (254 nm) and medium wave (310 nm) should not be used, the former being particularly damaging to nucleic acids.
2. The reaction must be done under reduced light. If desired, the lights can be turned off completely after the reaction has been set up.
3. Proper heat denaturation is critical for efficient labeling of DNA (see steps 2-3 on page 7). Make sure the heat denaturation is done at 100°C for at least 10 min. Quick-chill the sample *immediately* in a dry ice/alcohol bath. (Liquid nitrogen or an ice-water bath could be used instead.)
4. The pH of the nucleic acid solution must be between 2.5 and 10.
5. The salt concentration must be below 20 mM.

6. We have found that irradiation for as little as 15 min is often sufficient to produce high specific activity probes. We do not recommend irradiating the sample for more than an hour.

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### **C. Too Much Background in Hybridization Reactions**

The following suggestions may help reduce the background when using BrightStar Psoralen-Biotin labeled probe in blot hybridizations.

1. Filter the probe and hybridization solution before use.
2. Use no more than the probe concentrations recommended in section *III.D. Using BrightStar Psoralen-Biotin Labeled Probes* on page 9.
3. Increase the SDS concentration in the hybridization and wash buffers (up to a maximum of 2%).
4. Increase the blocking time prior to and after incubation with the antibody: conjugate solution.
5. Use a lower concentration of antibody:conjugate solution.
6. Increase the wash time and/or the number of washes after the conjugate binding and blocking steps.

## V. Appendix

### A. Nucleic Acid Clean-up and Precipitation Instructions

#### 1. DNase treatment

- a. Add 1–2 units of DNase I to the RNA and incubate at 37°C for 15 min.  
DNase is active in most solutions, use the reaction buffer provided with the enzyme, in vitro transcription buffer, or a restriction enzyme buffer with low to moderate salt concentrations, and some divalent cations.
- b. Inactivate DNase I by one of the following methods:
  - Add EDTA to 5 mM, heat to 75°C for 10 min
  - Add EDTA to 20 mM
- c. Treat with Proteinase K/SDS, phenol/chloroform extract, and precipitate the RNA (see below).

#### 2. Proteinase K/SDS treatment

This treatment degrades proteins in nucleic acid solutions

Incubate the nucleic acid with proteinase K (100–200 µg/mL) and 0.5% SDS for 30 min at 50°C, then do a phenol/chloroform extraction, and an ammonium acetate precipitation (see [3](#) and [4](#) below).

#### 3. Phenol/ chloroform extraction

Phenol/chloroform extraction removes proteins from nucleic acid solutions. For DNA, use ordinary buffer-saturated phenol/chloroform. For RNA, use either ordinary buffer-saturated phenol/chloroform or acid phenol/chloroform.

- a. Bring the volume of the nucleic acid solution to at least 100–200 µL with TE Buffer.
- b. Add an equal volume of phenol/chloroform (or acid phenol/chloroform for RNA), shake vigorously for ~30 seconds.
- c. Microfuge 5 min at top speed.
- d. Remove the aqueous (top) layer containing the nucleic acid to a fresh tube being careful to avoid transferring the interphase.
- e. Do an ammonium acetate precipitation (see [4](#) below).

#### 4. Ammonium acetate precipitation of DNA or RNA

Isopropanol precipitation is often preferable to ethanol precipitation because it precipitates fewer free nucleotides than ethanol.

- a. Add 1/10th volume 5 M ammonium acetate to the nucleic acid solution for a final concentration of 0.5 M NH<sub>4</sub>Ac.
- b. Add 1 volume of isopropanol (molecular biology grade) and mix well. Alternatively use 2 volumes 100% ethanol instead of isopropanol.

- c. Chill for at least 15 min at  $-20^{\circ}\text{C}$ .
- d. Microfuge at  $4^{\circ}\text{C}$  for 15 min at maximum speed to pellet the nucleic acid.
- e. Carefully remove the supernatant and wash the pellet with 70% EtOH.
- f. Resuspend the nucleic acid in TE Buffer to 50 ng/ $\mu\text{L}$ .

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## B. Quality Control

A 4 kb plasmid containing a 1 kb insert of the GAPDH gene is labeled using BrightStar Psoralen-Biotin according to the procedure. Labeled probe (0.1–1 pg) can be detected using the Ambion BioDetect Kit. A signal at 40 ng total RNA is detected in a Northern hybridization.

### 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.

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## C. 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](http://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.
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