SurePrep™ RNA Cleanup and Concentration Kit

Product Cat. # BP2809-50

Instruction Manual

I. Introduction ........................................................................................................................................... 2
   A. Product Description
   B. Overview of Procedure
   C. Kit Specifications
   D. Advantages
   E. Kit Components
   F. Storage Conditions and Product Stability
   G. Precautions and Disclaimers
   H. Customer-Supplied Reagents and Equipment
   I. Working with RNA

II. RNA Cleanup Procedures .................................................................................................................. 6
   A. Equipment Preparation
   B. Protocol for RNA Cleanup and Concentration from Enzymatic Reactions or Non-Phenol/Guanidine-Based RNA Isolation Methods
   C. Protocol for RNA Cleanup and Concentration from Phenol/Guanidine-Based RNA (Trizol® or Tri® Reagent) Isolation Methods
   D. Assessing RNA Yield and Quality by UV Absorbance
   E. Assessing RNA Quality by Denaturing Gel Electrophoresis

III. Troubleshooting Guide ...................................................................................................................... 11

IV. Related Products ............................................................................................................................... 12
   A. Additional RNA Purification Kits
   B. Other Fisher BioReagents Functionally Tested for RNA Research

V. Appendix A ......................................................................................................................................... 13
   Optional DNA Removal in Solution Followed by RNA Cleanup and Concentration
I. Introduction

A. Product Description
The SurePrep™ RNA Cleanup and Concentration Kit provides a rapid method for the purification and concentration of up to 50 µg of RNA isolated using different methods including phenol/guanidine-based protocols, and from various upstream enzymatic reactions such as DNase treatment, labeling and in vitro transcription. The kit purifies all sizes of RNA, from large mRNA and ribosomal RNA to microRNA (miRNA) and small interfering RNA (siRNA). The RNA is preferentially purified from other reaction components such as proteins, RNases and nucleotides without the use of phenol or chloroform. The purified RNA is of the highest quality, and can be used in a number of downstream applications including end-point or quantitative reverse transcription PCR, Northern blotting, RNase protection, primer extension, and expression array assays.

B. Overview of Procedure
Purification is based on spin column chromatography using a proprietary resin as the separation matrix. The RNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The process involves first mixing the RNA samples or enzymatic reactions containing RNA with Binding Solution (see flow chart on page 5). Ethanol is then added and the mixture is loaded onto a spin-column. The SurePrep™ resin binds RNA in a manner that depends on ionic concentrations. Thus, only the RNA will bind to the column while the contaminating proteins and nucleotides will be removed in the flowthrough. The bound RNA is then washed three times with the provided Wash Solution in order to remove any remaining impurities, and the purified RNA is eluted with low ionic strength Elution Buffer. The SurePrep RNA Cleanup and Concentration Kit purifies RNA with minimal amounts of DNA contamination. An optional protocol is provided in Appendix A for maximum removal of residual DNA that may affect sensitive downstream applications such as quantitative PCR.

C. Kit Specifications

<table>
<thead>
<tr>
<th>Kit Specifications</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Column Binding Capacity</td>
<td>50 µg</td>
</tr>
<tr>
<td>Size of RNA Purified</td>
<td>All sizes, including small RNA (&lt;200nt)</td>
</tr>
<tr>
<td>Maximum Amount of Starting Material</td>
<td>50 µg of RNA</td>
</tr>
<tr>
<td>Minimum Elution Volume</td>
<td>20 µL</td>
</tr>
<tr>
<td>Time to Complete 10 Purifications</td>
<td>20 minutes</td>
</tr>
<tr>
<td>Average Recovery</td>
<td>&gt; 90%</td>
</tr>
</tbody>
</table>

D. Advantages
- Efficient RNA cleanup from various enzymatic reactions such as labeling, DNase treatment and in vitro transcription
- Cleanup of RNA isolated using different methods, including phenol/chloroform extractions
- Fast and easy processing using rapid spin-column format
- Suitable for all sizes of RNA, from large mRNA to microRNA (miRNA)
E. Kit Components

<table>
<thead>
<tr>
<th>Component</th>
<th>Cat. # BP2809-50 (50 preps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding Solution</td>
<td>2 x 20 mL</td>
</tr>
<tr>
<td>Wash Solution*</td>
<td>22 mL</td>
</tr>
<tr>
<td>RNA Elution Buffer</td>
<td>6 mL</td>
</tr>
<tr>
<td>Collection Tubes</td>
<td>50</td>
</tr>
<tr>
<td>Spin Columns</td>
<td>50</td>
</tr>
<tr>
<td>Elution Tubes (1.7 mL)</td>
<td>50</td>
</tr>
<tr>
<td>Product Insert</td>
<td>1</td>
</tr>
</tbody>
</table>

*Prior to addition of 50 mL 95% ethanol

F. Storage Conditions and Product Stability
All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 2 years in their unopened containers.

G. Precautions and Disclaimers
This kit is designed for research purposes only. It is not intended for human or diagnostic use.

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDS). The MSDS can be requested through our Customer Service Department.

H. Customer-Supplied Reagents and Equipment
You must have the following in order to use the SurePrep™ RNA Cleanup and Concentration Kit.

For RNA cleanup and concentration from enzymatic reactions or non-phenol/guanidine-based RNA isolation methods
- Benchtop microcentrifuge
- β-mercaptoethanol
- 95 - 100% ethanol

For RNA cleanup and concentration from phenol/guanidine-based RNA (Trizol or Tri Reagent) isolation methods
- Benchtop microcentrifuge
- 70% ethanol

I. Working with RNA
RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these enzymes.
• The RNA area should be located away from microbiological work stations
• Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination
• There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only
• All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water
• Clean all surfaces with commercially available RNase decontamination solutions
• When working with purified RNA samples, ensure that they remain on ice during downstream applications
Flow Chart
Procedure for Purifying RNA using Fisher’s SurePrep™ RNA Cleanup and Concentration Kit

RNA Sample

Add Binding Solution and Ethanol

Vortex to mix

Bind to column

SPIN

Wash three times with Wash Solution

SPIN

Elute RNA with Elution Buffer

SPIN

Purified Total RNA
II. RNA Cleanup Procedures

A. Equipment Preparation
All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

\[
RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5}) (r)}}
\]

where \( RCF \) = required gravitational acceleration (relative centrifugal force in units of g); \( r \) = radius of the rotor in cm; and \( RPM \) = the number of revolutions per minute required to achieve the necessary g-force.

If you do not own a variable speed microcentrifuge consider purchasing Thermo Scientific’s Sorvall Legend benchtop model that offers power, safety and convenience.

- Choice of 17,000 or 21,000 x g (for RNA purification using SurePrep kits, the microcentrifuge with 17,000 x g is sufficient)
- Holds 36 x 0.5 mL microtubes, 24 x 2 mL tubes or 8 x 8 PCR
- Unique ClickSeal™ bio-containment rotor lid for safe processing of infectious specimens plus adequate clearance of SurePrep spin columns
- Fast acceleration and deceleration speeds up your protocols
- Broad range of rotors supports virtually any application
- Intuitive controls and vivid display
- Highly resistant materials allow vigorous cleaning and autoclaving

**Sorvall Legend MicroCentrifuges**

**Technical Specifications**

<table>
<thead>
<tr>
<th></th>
<th>Sorvall Legend Micro 17 &amp; 17R</th>
<th>Sorvall Legend Micro 21 &amp; 21R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max g-force:</td>
<td>17,000</td>
<td>21,100</td>
</tr>
<tr>
<td>Max RPM:</td>
<td>13,300</td>
<td>14,800</td>
</tr>
<tr>
<td>Noise level:</td>
<td>&lt;55 dBA</td>
<td>&lt;56 dBA</td>
</tr>
<tr>
<td>Time set range:</td>
<td>1 min - 99 min; 1 min increments</td>
<td>1 min - 99 min; 1 min increments</td>
</tr>
<tr>
<td>Temp set range:</td>
<td>Set from -9 °C to +40 °C; per 1 °C increment</td>
<td>Set from -9 °C to +40 °C; per 1 °C increment</td>
</tr>
</tbody>
</table>

**Ordering Information**

<table>
<thead>
<tr>
<th>Model</th>
<th>Cat. No.</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sorvall Legend Micro 17/17R</strong></td>
<td>230V 50/60Hz</td>
<td>120V 60 Hz</td>
</tr>
<tr>
<td>Sorvall Legend Micro 17, includes 24 x 1.5/2.0 mL rotor with ClickSeal bio-containment lid</td>
<td>75002430</td>
<td>75002431</td>
</tr>
<tr>
<td>Sorvall Legend Micro 17R, includes 24 x 1.5/2.0 mL rotor with ClickSeal bio-containment lid</td>
<td>75002440</td>
<td>75002441</td>
</tr>
<tr>
<td><strong>Sorvall Legend Micro 21/21R</strong></td>
<td>230V 50/60Hz</td>
<td>120V 60 Hz</td>
</tr>
<tr>
<td>Sorvall Legend Micro 21, incl. 24 x 1.5/2.0 mL rotor with ClickSeal bio-containment lid</td>
<td>75002435</td>
<td>75002436</td>
</tr>
<tr>
<td>Sorvall Legend Micro 21R, incl. 24 x 1.5/2.0 mL rotor with ClickSeal bio-containment lid</td>
<td>75002445</td>
<td>75002446</td>
</tr>
</tbody>
</table>

For detailed product specifications, information on additional rotors, lids, and adapters please visit [www.thermo.com](http://www.thermo.com).
B. Protocol for RNA Cleanup and Concentration from Enzymatic Reactions or Non-Phenol/Guanidine-Based RNA Isolation Methods

All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~12,000 RPM) except where noted. All centrifugation steps are performed at room temperature. Please be advised that all RPM values referenced in the protocols next to the actual g force are calculated for Sorvall Legend Microcentrifuge with rotor for 24 x 2 mL tubes.

Notes Prior to Use

- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the Wash Solution by adding 50 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated Wash Solution. This will give a final volume of 72 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Prepare an appropriate amount of Binding Solution by adding 10 μL of β-mercaptoethanol (provided by the user) to each 1 mL of Binding Solution required. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
- It is recommended that no more than 50 μg of RNA is used per cleanup.
- The maximum volume of RNA sample that can be processed is 200 μL.
- It is important to work quickly during this procedure.
- This kit purifies RNA with minimal amounts of DNA contamination. However, an optional protocol is provided in Appendix A for maximum removal of residual DNA that may affect sensitive downstream applications such as quantitative PCR. The procedure in Appendix A is to be carried out prior to performing the kit procedure below.

1. Sample Preparation

   a. Adjust the volume of the RNA sample to 100 μL by adding RNase-free or DEPC-treated water. It is recommended that no more than 50 μg of RNA be used for each column.

      Note: If an input volume between 100 and 200 μL is used, adjust the sample volume to 200 μL (maximum allowable) with RNase-free or DEPC-treated water. In this case, use the volumes indicated in bold in the bracket in Steps 1b and 1c.

   b. Add 250 μL (or 500 μL) of Binding Solution to the RNA sample. Mix by vortexing
   c. Add 200 μL (or 400 μL) of 95 – 100% ethanol (provided by the user) to the mixture from step 1b. Mix by vortexing for 10 seconds.

2. Binding to Column

   a. Assemble a column with one of the provided collection tubes.
   b. Apply up to 600 μL of the RNA sample with the ethanol (from Step 1c) onto the column and centrifuge for 1 minute.
   c. Discard the flowthrough. Reassemble the spin column with its collection tube.
   d. If the volume of the RNA sample is greater than 600 μL, repeat Step 2b and 2c until all the remaining RNA sample has passed through the column.

3. Column Wash

   a. Apply 400 μL of Wash Solution to the column and centrifuge for 1 minute.
Note: Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Repeat steps 3a and 3b to wash the column a second time.
d. Wash column a third time by adding another 400 μL of Wash Solution and centrifuging for 2 minutes.
e. Ensure that the column is dry. Spin for an additional minute, if necessary.
f. Discard the collection tube with the flowthrough.

4. RNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 50 μL of Elution Buffer to the column.

Note: For higher concentrations of RNA, a lower elution volume may be used. A minimum volume of 20 μL is recommended

c. Centrifuge for 2 minutes at 200 x g (~1,500 RPM), followed by 1 minute at 14,000 x g (~12,000 RPM) Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at 14,000 x g (~12,000 RPM) for 1 additional minute.

Note: For maximum RNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat Steps 4b and 4c).

5. Storage of RNA

The purified RNA sample may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

C. Protocol for RNA Cleanup and Concentration from Phenol/Guanidine-Based RNA (TRIzol1 or Tri1 Reagent) Isolation Methods

All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~12,000 RPM) except where noted. All centrifugation steps are performed at room temperature.

Notes Prior to Use

• Ensure that all solutions are at room temperature prior to use.
• Prepare a working concentration of the Wash Solution by adding 50 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated Wash Solution. This will give a final volume of 72 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
• It is recommended that no more than 50 μg of RNA is used per cleanup.
• It is important to work quickly during this procedure.

1. Sample Preparation

a. Isolate RNA using a phenol/guanidine-based reagent such as Trizol or Tri Reagent according to manufacturer’s instruction. After the separation of the aqueous and organic phases, collect the upper (aqueous) fractions containing the RNA into a new RNase-free microcentrifuge tube (not provided). Note the volume.
b. Add one volume of 70% ethanol (provided by the user) to the fraction from step 1a. Mix by vortexing for 10 seconds.

2. Binding to Column
   a. Assemble a column with one of the provided collection tubes.
   b. Apply up to 600 μL of the RNA mixed with the ethanol (from Step 1b) onto the column and centrifuge for 1 minute.
   c. Discard the flowthrough. Reassemble the spin column with its collection tube.
   d. If the volume of RNA mix is greater than 600 μL, repeat Steps 2b and 2c until all the remaining RNA mix has passed through the column.

3. Column Wash
   a. Apply 400 μL of Wash Solution to the column and centrifuge for 1 minute.
      
      **Note:** Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.
   b. Discard the flowthrough and reassemble the spin column with its collection tube.
   c. Repeat steps 3a and 3b to wash the column a second time.
   d. Wash column a third time by adding another 400 μL of Wash Solution and centrifuging for 2 minutes.
   e. Ensure that the column is dry. Spin for an additional minute, if necessary.
   f. Discard the collection tube with the flowthrough.

4. RNA Elution
   a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
   b. Add 50 μL of Elution Buffer to the column.
      
      **Note:** For higher concentrations of RNA, a lower elution volume may be used. A minimum volume of 20 μL is recommended.
   c. Centrifuge for 2 minutes at 200 x g (~1,5000 RPM), followed by 1 minute at 14,000 x g (~12,000 RPM) Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at 14,000 x g (~12,000 RPM) for one additional minute.
      
      **Note:** For maximum RNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat Steps 4b and 4c).

5. Storage of RNA
   The purified RNA sample may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

¹Trizol is a trademark of Invitrogen. Tri Reagent is a trademark of Sigma-Aldrich.
D. Assessing RNA Yield and Quality by UV Absorbance

The concentration and purity of an RNA solution can be determined by absorbance (A) measurements at 260 and 280 nm. A$_{260}$ measurements are quantitative for relatively pure RNA preparations in microgram quantities. A$_{260}$ readings cannot distinguish between DNA and RNA, however the ratio of A$_{260}$/A$_{280}$ can be used as an indication of RNA purity. For example, contaminating proteins have a peak absorption at 280 nm that will reduce the A$_{260}$/A$_{280}$ ratio.

a. Determine RNA concentration by diluting an aliquot of the purified RNA solution (e.g. 1:50 dilution) in TE (10 mM Tris and 1 mM EDTA, pH 7.4). Measure absorbance of the diluted sample in a 1 mL cuvette using a traditional UV-VIS spectrophotometer at 260 and 280 nm. The spectrophotometer should first be zeroed with the TE used to dilute the sample.

b. An A$_{260}$ of 1.0 is equivalent to 40 µg RNA/mL. Calculate the RNA concentration in µg/mL as follows:

$$A_{260} \times \text{dilution factor} \times 40 = \mu g \text{ RNA/mL}$$

c. The ratio of the readings at 260 and 280 nm (A$_{260}$/A$_{280}$) provides an estimate of the RNA purity with respect to contaminants that absorb in the UV range such as protein. Ratios of 1.8 to 2.1 indicate highly purified preparations of RNA. Contaminants such as protein that absorb at 280 nm will lower this ratio. However, RNA solutions with a ratio lower than 1.8 may function well in downstream applications such as RT-PCR and Northern blotting.

E. Assessing RNA Quality by Denaturing Gel Electrophoresis

The overall quality and size distribution of total RNA purified with the SurePrep™ Kit can be evaluated by denaturing gel electrophoresis with ethidium bromide staining or by using the Agilent 2100 Bioanalyzer.
Figure 1. Total RNA from *E. coli* was isolated using the phenol/guanidine-based Tri Reagent Method. The isolated RNA was then cleaned and concentrated using the SurePrep™ Kit and loaded in lanes 2 and 3 on a 1.5% denaturing formaldehyde-agarose gel. Lane 1 contained a 1 kb RNA standard (BP2811). Electrophoresis was performed at 100 V for 70 min and the gel stained with ethidium bromide.
### III. Troubleshooting Guide

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution and Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor RNA Recovery</td>
<td>Column has become clogged</td>
<td>Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. See also “Clogged Column” below.</td>
</tr>
<tr>
<td></td>
<td>An alternative elution solution was used</td>
<td>It is recommended that the Elution Buffer supplied with this kit be used for maximum RNA recovery.</td>
</tr>
<tr>
<td></td>
<td>Ethanol was not added to the lysate</td>
<td>Ensure that the appropriate amount of ethanol is added to the lysate before binding to the column.</td>
</tr>
<tr>
<td></td>
<td>Ethanol was not added to the Wash Solution</td>
<td>Ensure that 50 mL of 95% ethanol is added to the supplied Wash Solution prior to use.</td>
</tr>
<tr>
<td>Clogged Column</td>
<td>High amounts of RNA in the input</td>
<td>Ensure that no more than 50 μg of RNA is used as the input for each column.</td>
</tr>
<tr>
<td></td>
<td>High amounts of genomic DNA present in sample</td>
<td>The lysate may be passed through a 25 gauge needle attached to a syringe 5-10 times in order to shear the genomic DNA prior to loading onto the column.</td>
</tr>
<tr>
<td></td>
<td>Centrifuge temperature too low</td>
<td>Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 20°C may cause precipitates to form that can cause the columns to clog.</td>
</tr>
<tr>
<td>RNA is Degraded</td>
<td>RNase contamination</td>
<td>RNases may be introduced during the use of the kit. Ensure proper procedures are followed when working with RNA. Please refer to “Working with RNA” at the beginning of this user guide.</td>
</tr>
<tr>
<td></td>
<td>Procedure not performed quickly enough</td>
<td>In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly.</td>
</tr>
<tr>
<td></td>
<td>Improper storage of the purified RNA</td>
<td>For short term storage RNA samples may be stored at −20°C for a few days. It is recommended that samples be stored at −70°C for longer term storage.</td>
</tr>
<tr>
<td>Problem</td>
<td>Possible Cause</td>
<td>Solution and Explanation</td>
</tr>
<tr>
<td>---------</td>
<td>---------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>RNA does not perform well in downstream applications</td>
<td>RNA was not washed three times with the provided Wash Solution</td>
<td>Traces of salt from the binding step may remain in the sample if the column is not washed three times with the Wash Solution. Salt may interfere with downstream applications, and thus must be washed from the column.</td>
</tr>
<tr>
<td>DNA or Genomic DNA contamination</td>
<td>Large amounts of starting material used</td>
<td>Perform RNAse-free DNaseI digestion on the RNA sample after elution to remove DNA or genomic DNA contamination.</td>
</tr>
<tr>
<td>Ethanol carryover</td>
<td></td>
<td>Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.</td>
</tr>
</tbody>
</table>

### IV. Related Products

#### A. Additional RNA Purification Kits

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Product Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP2800-50</td>
<td>SurePrep™ TrueTotal™ RNA Purification Kit</td>
</tr>
<tr>
<td>BP2801-25</td>
<td>SurePrep™ Small RNA Purification Kit</td>
</tr>
<tr>
<td>BP2802-50</td>
<td>SurePrep™ RNA/DNA/Protein Purification Kit</td>
</tr>
<tr>
<td>BP2803-50</td>
<td>SurePrep™ Urine Exfoliated Cell RNA Purification Kit</td>
</tr>
<tr>
<td>BP2804-50</td>
<td>SurePrep™ Urine Bacterial RNA Purification Kit</td>
</tr>
<tr>
<td>BP2805-50</td>
<td>SurePrep™ Nuclear Or Cytoplasmic RNA Purification Kit</td>
</tr>
<tr>
<td>BP2806-50</td>
<td>SurePrep™ RNA/Protein Purification Kit</td>
</tr>
<tr>
<td>BP2807-50</td>
<td>SurePrep™ Leukocyte RNA Purification Kit</td>
</tr>
<tr>
<td>BP2809-50</td>
<td>SurePrep™ RNA Cleanup and Concentration Kit</td>
</tr>
</tbody>
</table>

#### B. Other Fisher BioReagents Functionally Tested for RNA Research

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Product Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP2484-50</td>
<td>Water, Sterile (DEPC-treated) 50mL</td>
</tr>
<tr>
<td>BP2484-100</td>
<td>Water, Sterile (DEPC-treated) 100mL</td>
</tr>
<tr>
<td>BP561-1</td>
<td>Water, Sterile (RNA Grade) 1L</td>
</tr>
<tr>
<td>BP2483-100</td>
<td>EDTA 0.5 M (DEPC-treated) 100mL</td>
</tr>
<tr>
<td>BP2483-1</td>
<td>EDTA 0.5 M (DEPC-treated) 1L</td>
</tr>
<tr>
<td>BP2483-500</td>
<td>EDTA 0.5 M (DEPC-treated) 500mL</td>
</tr>
<tr>
<td>BP2810-50</td>
<td>RiboLadder™ 100b RNA Standard with loading buffers</td>
</tr>
<tr>
<td>BP2811-50</td>
<td>RiboLadder™ 1 Kb RNA Standard with loading buffers</td>
</tr>
<tr>
<td>BP3224-5</td>
<td>Optizyme™ Ribonuclease Inhibitor (Human Placental) 10,000U</td>
</tr>
<tr>
<td>BP3224-1</td>
<td>Optizyme™ Ribonuclease Inhibitor (Human Placental) 2,500U</td>
</tr>
<tr>
<td>BP3225-5</td>
<td>Optizyme™ Ribonuclease Inhibitor (Porcine) 10,000U</td>
</tr>
<tr>
<td>BP3225-1</td>
<td>Optizyme™ Ribonuclease Inhibitor (Porcine) 2,500U</td>
</tr>
</tbody>
</table>
V. Appendix A

Optional DNA Removal in Solution Followed by RNA Clean-Up and Concentration

The SurePrep™ RNA Cleanup and Concentration Kit purifies RNA with minimal amounts of DNA contamination. An optional protocol is provided below for maximum removal of residual DNA that may affect sensitive downstream applications such as quantitative PCR. It is recommended that an RNase-free DNase I be used. This procedure is to be performed prior to starting the kit protocol.

1. Adjust the volume of the RNA sample to be treated to 80 μL with RNase-free water.

2. Prepare a working stock of 0.5 Kunitz unit/μL RNase-free DNase I solution according to the manufacturer’s instructions. Alternatively, dissolve or dilute stock DNase I in a reaction buffer (40 mM Tris pH 8.0, 10 mM MgCl₂ and 3 mM CaCl₂, made RNase-free) to give a final concentration of 0.5 Kunitz unit/μL.

3. Add 20 μL of 0.5 Kunitz unit/μL DNase I to the RNA sample.

4. Incubate at 25 to 30°C for 15 minutes.

5. Proceed directly to Protocol A “Protocol for RNA Clean-up and Concentration from Enzymatic Reactions or Non-Phenol/Guanidine-Based RNA Isolation Methods”.

Technical Support

Telephone: 1-800-227-6701
Email: chem..techinfo@thermofisher.com

© 2007 Fisher BioReagents®