Fisher BioReagents®

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SurePrep™ RNA Cleanup and Concentration Kit

Product Cat. # BP2809-50

Instruction Manual

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

Kit Specifications	
Column Binding Capacity	50 μg
Size of RNA Purified	All sizes, including small RNA (<200nt)
Maximum Amount of Starting Material	50 μg of RNA
Minimum Elution Volume	20 μL
Time to Complete 10 Purifications	20 minutes
Average Recovery	≥ 90%

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

Component	Cat. # BP2809-50 (50 preps)
Binding Solution	2 x 20 mL
Wash Solution*	22 mL
RNA Elution Buffer	6 mL
Collection Tubes	50
Spin Columns	50
Elution Tubes (1.7 mL)	50
Product Insert	1

*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

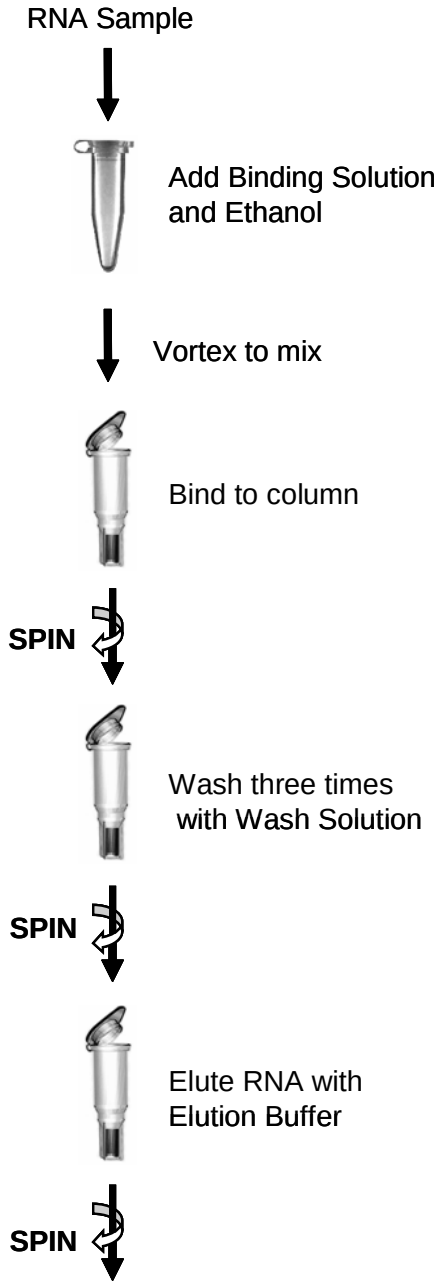
I. Working with RNA

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



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

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

Ordering Information

	Cat. No.	Cat. No.
Sorvall Legend Micro 17/17R	230V 50/60Hz	120V 60 Hz
Sorvall Legend Micro 17, includes 24 x 1.5/2.0 mL rotor with ClickSeal bio-containment lid	75002430	75002431
Sorvall Legend Micro 17R, includes 24 x 1.5/2.0 mL rotor with ClickSeal bio-containment lid	75002440	75002441
Sorvall Legend Micro 21/21R	230V 50/60Hz	120V 60 Hz
Sorvall Legend Micro 21, incl. 24 x 1.5/2.0 mL rotor with ClickSeal bio-containment lid	75002435	75002436
Sorvall Legend Micro 21R, incl. 24 x 1.5/2.0 mL rotor with ClickSeal bio-containment lid	75002445	75002446

For detailed product specifications, information on additional rotors, lids, and adapters please visit 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 (TRIzol[1] or Tri[1] 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.

[1]Trizol 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} x dilution factor x 40 = µg 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.

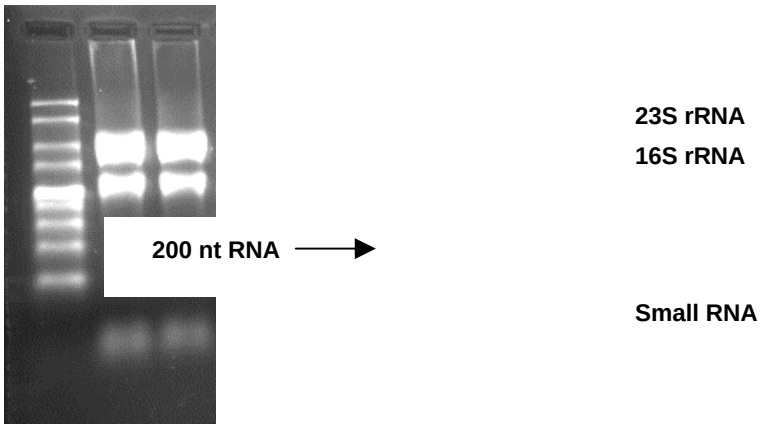


Figure 1. Total RNA from *E. coli* was isolated using the phenol/guanidine-based Tri Reagent Method. The isolated RNA was then cleaned and concentrated using the SurePrep™ Kit and loaded in lanes 2 and 3 on a 1.5% denaturing formaldehyde-agarose gel. Lane 1 contained a 1 kb RNA standard (BP2811). Electrophoresis was performed at 100 V for 70 min and the gel stained with ethidium bromide.

III. Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Column has become clogged	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.
	An alternative elution solution was used	It is recommended that the Elution Buffer supplied with this kit be used for maximum RNA recovery.
	Ethanol was not added to the lysate	Ensure that the appropriate amount of ethanol is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution	Ensure that 50 mL of 95% ethanol is added to the supplied Wash Solution prior to use.
Clogged Column	High amounts of RNA in the input	Ensure that no more than 50 μg of RNA is used as the input for each column.
	High amounts of genomic DNA present in sample	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.
	Centrifuge temperature too low	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.
RNA is Degraded	RNase contamination	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.
	Procedure not performed quickly enough	In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly.
	Improper storage of the purified RNA	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.

Problem	Possible Cause	Solution and Explanation
RNA does not perform well in downstream applications	RNA was not washed three times with the provided Wash Solution	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.
	Ethanol carryover	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.
DNA or Genomic DNA contamination	Large amounts of starting material used	Perform RNAse-free DNaseI digestion on the RNA sample after elution to remove DNA or genomic DNA contamination.

IV. Related Products

A. Additional RNA Purification Kits

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

B. Other Fisher BioReagents Functionally Tested for RNA Research

BP2484-50	Water, Sterile (DEPC-treated) 50mL
BP2484-100	Water, Sterile (DEPC-treated) 100mL
BP561-1	Water, Sterile (RNA Grade) 1L
BP2483-100	EDTA 0.5 M (DEPC-treated) 100mL
BP2483-1	EDTA 0.5 M (DEPC-treated) 1L
BP2483-500	EDTA 0.5 M (DEPC-treated) 500mL
BP2810-50	RiboLadder™ 100b RNA Standard with loading buffers
BP2811-50	RiboLadder™ 1Kb RNA Standard with loading buffers
BP3224-5	Optizyme™ Ribonuclease Inhibitor (Human Placental) 10,000U
BP3224-1	Optizyme™ Ribonuclease Inhibitor (Human Placental) 2,500U
BP3225-5	Optizyme™ Ribonuclease Inhibitor (Porcine) 10,000U
BP3225-1	Optizyme™ Ribonuclease Inhibitor (Porcine) 2,500U

BP3222-5	Optizyme™ Ribonuclease Inhibitor (Recombinant) 10,000U
BP3222-1	Optizyme™ Ribonuclease Inhibitor (Recombinant) 2,500U
BP3226-1	Optizyme™ Recombinant DNase I (RNase-free) 1,000U
BP3226-2	Optizyme™ Recombinant DNase I (RNase-free) 2,000U
BP176-100	2-Mercaptoethanol 100g
BP535-1	Lysozyme, Egg White 1g
BP535-5	Lysozyme, Egg White 5g
BP535-10	Lysozyme, Egg White 10g
BP2476-100	Tris-EDTA, 1X Solution, pH 7.4 100ml
BP2476-500	Tris-EDTA, 1X Solution, pH 7.4 500ml
BP160-100	Agarose, Low EEO, Multipurpose 100g
BP1360-100	Agarose, Low Melting, <1kb RNA 100g
BP1356-100	Agarose, Broad Separation Range for RNA 100g
BP1302-10	Ethidium Bromide 1% Solution 10ml
BP102-1	Ethidium Bromide 1g

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 quantitative PCR. It is recommended that an RNase-free DNase I be used. This procedure is to be preformed 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 $MgCl_2$ and 3 mM $CaCl_2$, 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