Fisher BioReagents®

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SurePrep™ TrueTotal™ RNA Purification Kit

Product Cat. # BP2800-50

Instruction Manual

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I. Introduction

A. Product Description

As RNA analysis gains the spotlight for assessing the functional characterization of disease-resistant genes for drug discovery and basic research, there is a growing need for more precise and reliable tools for extracting and purifying RNA molecules. The SurePrep™ TrueTotal™ RNA Purification Kit provides a rapid method for the isolation and purification of total RNA from cultured animal cells, tissue samples, blood, bacteria, yeast, fungi and plants. The kit purifies **all sizes** of RNA, from large mRNA and ribosomal RNA down to micro RNA (miRNA) and small interfering RNA (siRNA). The RNA is preferentially purified from other cellular macromolecules such as DNA and proteins without the use of phenol or chloroform. The purified intact RNA is of the highest quality, and can be used in a number of downstream applications such as quantitative RT-PCR, Northern blotting, RNase protection, and expression array assays.

B. Overview of Procedure

Purification is based on spin column chromatography using a proprietary resin as the separation matrix. Biological samples are first disrupted in a highly denaturing guanidine thiocyanate Lysis Solution that simultaneously lyses cells and inactivates endogenous ribonucleases to ensure purification of intact RNA (flow chart on page 5). The lysate is diluted with ethanol to provide appropriate binding conditions, and the sample is then applied to a SurePrep spin column where the total RNA binds to the resin and most other cellular contaminants are effectively washed away. Up to 50 µg of high quality total RNA is then eluted in 50 µL of low ionic strength Elution Buffer.

C. Kit Specifications

Kit Specifications	
Column Binding Capacity	50 µg
Maximum Column Loading Volume	600 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Maximum Amount of Starting Material: Animal Cells Animal Tissues Blood Bacteria Yeast Fungi Plant Tissues	 3×10^6 cells 25 mg 100 µL 1×10^9 cells 1×10^8 cells 50 mg 50 mg
Time to Complete Ten Purifications	20 minutes
Average Yields* HeLa Cells (1×10^6 cells) *E. coli* (1×10^9 cells)	 15 µg 50 µg

* *Average yields will vary depending upon a number of factors including species, growth conditions, and developmental stage.*

D. Advantages

- Fast and easy processing using rapid spin-column format
- Isolate all sizes of RNA including large RNA and small RNA species
- No phenol or chloroform extractions
- Isolate high quality total RNA from a variety of biological sources
- RNA can be isolated and detected from as little as a single animal cell

E. Kit Components

Component	Catalog # BP2800-50 (50 preps)
Lysis Solution	40 mL
Wash Solution*	22 mL
Elution Buffer	6 mL
Micro Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

***Before addition of 50 mL 95% ethanol**

F. Storage Conditions and Product Stability

All kit 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.

Blood of all human and animal subjects are considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with whole blood.

H. Customer-Supplied Reagents and Equipment

You must have the following in order to use the TrueTotal RNA Purification Kit:

For All Protocols

- Benchtop microcentrifuge
- 95 - 100% ethanol
- β-mercaptoethanol (optional)

For Animal Cell Protocol

- PBS (RNase-free)

For Animal Tissue Protocol

- Liquid nitrogen
- Mortar and pestle
- 70% ethanol

For Bacterial Protocol

- Lysozyme-containing TE Buffer:
    - For Gram-negative bacteria, 1 mg/mL lysozyme in TE Buffer
    - For Gram-positive bacteria, 3 mg/mL lysozyme in TE Buffer

For Yeast Protocol

- Resuspension Buffer with Lyticase:
    - 50 mM Tris pH 7.5
    - 10 mM EDTA
    - 1 M Sorbital
    - 1 unit/μL Lyticase

For Fungi Protocol

- Liquid nitrogen
- Mortar and pestle
- 70% ethanol

For Plant Protocol

- Liquid nitrogen
- Mortar and pestle
- 70% ethanol

I. Working with RNA

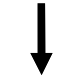
RNases are stable, robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to inactivate these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these 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

Flowchart

Procedure for TrueTotal RNA Purification Kit

Lyse cells or tissue using **Lysis Solution**



Add Ethanol

Centrifuge to clear lysate

(Optional)

Bind to column

SPIN

Wash three times with Wash Solution

SPIN

Elute RNA with Elution Buffer

SPIN

Purified Total RNA

II. Set-Up and Preparation of Sample Lysate

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
- 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 Micro Centrifuges

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 LegendMicro 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 visit **www.thermo.com**

B. Preparation of Lysate from Various Cell Types

Notes Prior to Use

- The steps for preparing the lysate are different depending on the starting material (Section II C-I). However, the subsequent steps are the same in all cases (Section III A-C).
- Please ensure that the correct procedure for preparing the lysate from your starting material is followed.
- 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.
- 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.
- **Optional:** The use of β-mercaptoethanol in lysis is highly recommended for most animal tissues, particularly those known to have a high RNAse content (e.g. pancreas), as well as for most plant tissues. It is also recommended for users who wish to isolate RNA for sensitive downstream applications. Add 10 μL of β-mercaptoethanol (provided by the user) to each 1 mL of Lysis Solution required. β-mercaptoethanol is toxic and should be dispensed in a fume hood. Alternatively, the lysis solution can be used as provided.
- It is important to work quickly during this procedure.
- 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 2mL tubes.

C. Lysate Preparation from Cultured Animal Cells

Notes Prior to Use

- The maximum recommended input of cells is 3×10^6. A hemocytometer can be used in conjunction with a microscope to count the number of cells. As a general guideline, a confluent 3.5 cm plate of HeLa cells will contain 10^6 cells.
- Cell pellets can be stored at -70°C for later use or used directly in the procedure. Determine the number of cells present before freezing.
- Frozen pellets should be stored for no longer than 2 weeks to ensure that the integrity of the RNA is not compromised.
- Frozen cell pellets should not be thawed prior to beginning the protocol. Add the Lysis Solution directly to the frozen cell pellet.

Cell Lysate Preparation from Cells Growing in a Monolayer

a. Aspirate media and wash cell monolayer with an appropriate amount of PBS. Aspirate PBS.
b. Add 350 μL of **Lysis Solution** directly to culture plate.
c. Lyse cells by gently tapping culture dish and swirling buffer around plate surface for five minutes.
d. Transfer lysate to a microcentrifuge tube.
e. Add 200 μL of 95 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.

Note: For input amounts greater than 10^6 cells, it is recommended that the lysate is passed through a 25 gauge needle attached to a syringe 5-10 times at this point, in order to shear the genomic DNA prior to loading onto the column.

f. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to Section IIIA without centrifugation.

Cell Lysate Preparation from Cells Growing in Suspension and Lifted Cells

a. Transfer cell suspension to an RNase-free tube (not provided) and centrifuge at no more than 200 *x g* (~1,500 RPM) for 10 minutes to pellet cells.
b. Carefully decant the supernatant. A few μL of media may be left behind with the pellet in order to ensure that the pellet is not dislodged.
c. Add 350 μL of **Lysis Solution** to the pellet. Lyse cells by vortexing for 15 seconds. Ensure that the entire pellet is completely dissolved before proceeding to the next step.
d. Add 200 μL of 95 - 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.

Note: For input amounts greater than 10^6 cells, it is recommended that the lysate is passed through a 25 gauge needle attached to a syringe 5-10 times at this point, in order to shear the genomic DNA prior to loading onto the column.

e. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to Section IIIA without centrifugation.

D. Lysate Preparation from Animal Tissues

Notes Prior to Use

- RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized. Thus it is important that the procedure is carried out as quickly as possible, particularly the Cell Lysate Preparation step.
- Fresh or frozen tissues may be used for the procedure. Tissues should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Tissues may be stored at -70°C for several months. When isolating total RNA from frozen tissues ensure that the tissue does not thaw during weighing or prior to grinding with the mortar and pestle.
- It is recommended that no more than 25 mg of tissue be used, in order to prevent clogging of the column.

Cell Lysate Preparation from Animal Tissues

a. Excise the tissue sample from the animal.
b. Determine the amount of tissue by weighing. It is recommended that no more than 25 mg of tissue be used for the protocol.
c. Transfer the tissue into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the tissue thoroughly using a pestle.
d. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
e. Add 600 μL of **Lysis Solution** to the tissue sample and continue to grind until the sample has been homogenized. Homogenize by passing the lysate 5-10 times through a 25 gauge needle attached to a syringe.
f. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).

g. Spin the lysate for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube. Note the volume of the supernatant/lysate.
h. Add an equal volume of 70% ethanol (provided by the user) to the lysate volume collected (100 μL of ethanol is added to every 100 μL of lysate). Vortex to mix.
i. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to Section IIIA without centrifugation.

E. Lysate Preparation from Blood

Notes Prior to Use

- Blood of all human and animal subjects are considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with whole blood.
- It is recommended that no more than 100 μL of blood be used in order to prevent clogging of the column.
- We recommend the use of this kit to isolate RNA from non-coagulating fresh blood using EDTA as the anti-coagulant.
- It is important to work quickly during this procedure.

Cell Lysate Preparation from Blood

a. Transfer up to 100 μL of non-coagulating blood to an RNase-free microcentrifuge tube (not provided).
b. Add 350 μL of **Lysis Solution** to the blood. Lyse cells by vortexing for 15 seconds. Ensure that mixture becomes transparent before proceeding to the next step.
c. Add 200 μL of 95 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.
d. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to Section IIIA without centrifugation.

F. Lysate Preparation from Bacteria

Notes Prior to Use

- Prepare the appropriate lysozyme-containing TE Buffer as indicated in Table 1. This solution should be prepared with sterile, RNAse-free TE Buffer, and kept on ice until needed. These reagents are to be provided by the user.
- It is recommended that no more than 10^9 bacterial cells be used in this procedure. Bacterial growth can be measured using a spectrophotometer. As a general rule, an *E. coli* culture containing 1 x 10^9 cells/mL has an OD_{600} of 1.0.
- For RNA isolation, bacteria should be harvested in log-phase growth.
- Bacterial pellets can be stored at -70°C for later use, or used directly in this procedure.
- Frozen bacterial pellets should not be thawed prior to beginning the protocol. Add the Lysozyme-containing TE Buffer directly to the frozen bacterial pellet.

Cell Lysate Preparation from Bacteria

a. Pellet bacteria by centrifuging at 14,000 *x g* (~12,000 RPM) for 1 minute.
b. Decant supernatant, and carefully remove any remaining media by aspiration.

c. Resuspend the bacteria thoroughly in 100 µL of the appropriate lysozyme-containing TE buffer (see Table 1) by vortexing. Incubate at room temperature for the time indicated in Table 1.

Table 1: Incubation Time for Different Bacterial Strains

Bacteria Type	Lysozyme Concentration in TE Bufffer	Incubation Time
Gram-negative	1 mg/mL	5 min
Gram-positive	3 mg/mL	10 min

d. Add 300 µL of Lysis Solution and vortex vigorously for at least 10 seconds.
e. Add 200 µL of 95 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.
f. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to Section IIIA without centrifugation.

G. Lysate Preparation from Yeast

Notes Prior to Use

- Prepare the appropriate amount of Lyticase-containing Resuspension Buffer, considering that 100 µL of buffer is required for each preparation. The Resuspension Buffer should have the following composition: 50 mM Tris, pH 7.5, 10 mM EDTA, 1M Sorbital, 0.1% β-mercaptoethanol and 1 unit/µL Lyticase. This solution should be prepared with sterile, RNAse-free reagents, and kept on ice until needed. These reagents are to be provided by the user.
- It is recommended that no more than 10^8 yeast cells or 1 mL of culture be used for this procedure.
- For RNA isolation, yeast should be harvested in log-phase growth.
- Yeast can be stored at -70°C for later use, or used directly in this procedure.
- Frozen yeast pellets should not be thawed prior to beginning the protocol. Add the Lyticase-containing Resuspension Buffer directly to the frozen yeast pellet.

Cell Lysate Preparation

a. Pellet yeast by centrifuging at 14,000 *x g* (~12,000 RPM) for 1 minute.
b. Decant supernatant, and carefully remove any remaining media by aspiration.
c. Resuspend the yeast thoroughly in 100 µL of Lyticase-containing Resuspension Buffer by vortexing. Incubate at 37°C for 10 minutes.
d. Add 300 µL of Lysis Solution and vortex vigorously for at least 10 seconds.
e. Add 200 µL of 95 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.
f. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to Section IIIA without centrifugation.

H. Lysate Preparation from Fungi

Notes Prior to Use

- Fresh or frozen fungi may be used for this procedure. Fungal tissues should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Fungi may be stored at -70°C for several months. Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised.
- It is recommended that no more than 50 mg of fungi be used for this procedure in order to prevent clogging of the column.
- It is important to work quickly during this procedure.

Cell Lysate Preparation from Fungi

a. Determine the amount of fungi by weighing. It is recommended that no more than 50 mg of fungi be used for the protocol.
b. Transfer the fungus into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the fungus thoroughly using a pestle.

   Note: At this stage the ground fungus may be stored at -70°C, such that the RNA purification can be performed at a later time.

c. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
d. Add 600 µL of **Lysis Solution** to the tissue sample and continue to grind until the sample has been homogenized.
e. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
f. Spin the lysate for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube. Note the volume of the supernatant/lysate.
g. Add an equal volume of 70% ethanol (provided by the user) that is equivalent to the lysate volume collected (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix.
h. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to Section IIIA without centrifugation.

I. Lysate Preparation from Plant

Notes Prior to Use

- The maximum recommended input of plant tissue is 50 mg or 5 x 10^6 plant cells.
- Both fresh and frozen plant samples can be used for this protocol. Samples should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised.
- It is important to work quickly during this procedure.

Cell Lysate Preparation from Plant

a. Transfer ≤50 mg of plant tissue or 5 x 10^6 plant cells into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the sample into a fine powder using a pestle in liquid nitrogen.

Note: If stored frozen samples are used, do not allow the samples to thaw before transferring to the liquid nitrogen.

b. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
c. Add 600 µL of **Lysis Solution** to the tissue sample and continue to grind until the sample has been homogenized.
d. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
e. Spin the lysate for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube. Note the volume of the supernatant/lysate.
f. Add an equal volume of 70% ethanol (provided by the user) that is equivalent to the lysate volume collected (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix.
g. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to Section IIIA without centrifugation.

III. Purifying Total RNA from Different Sample Lysates

Note: *The remaining steps in the purification of total RNA are the same from this point forward for all the different types of sample lysate.*

A. Binding RNA to Column

a. Assemble a column with one of the provided collection tubes
b. Apply up to 600 µL of the clarified lysate with the ethanol (from Section II) onto the column and centrifuge for 1 minute.
c. Discard the flowthrough. Reassemble the spin column with its collection tube.
d. Depending on your lysate volume, repeat Steps b and c above.

Optional Step:

Our TrueTotal RNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional **On-Column DNA Removal Protocol** is provided in Appendix A for maximum removal of residual DNA that may affect sensitive downstream RNA applications. This step should be performed at this point in the protocol.

B. 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 a/b to wash 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 flow-through.

C. 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.
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 50 µL 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 RNA elution be performed into a separate microcentrifuge tube (repeat steps b/c above).

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.

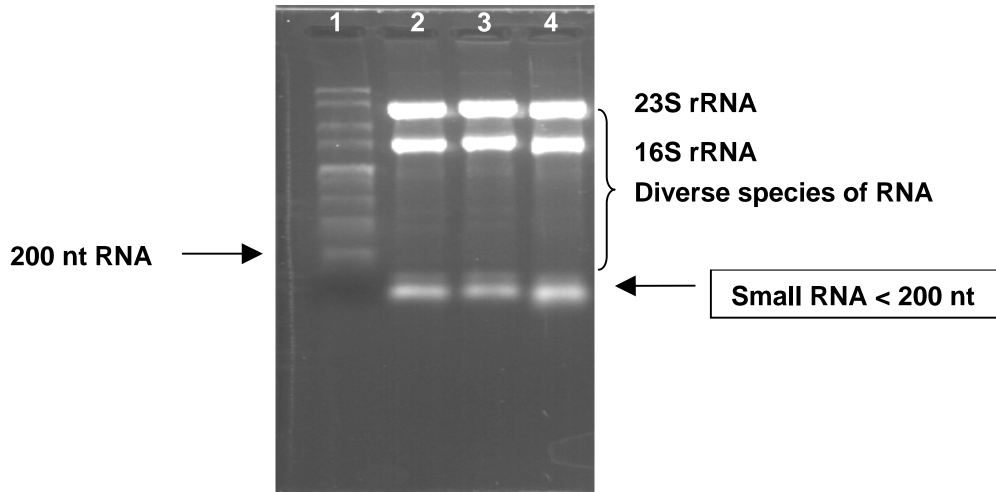
a. Determine RNA concentration by diluting an aliquot of the purified RNA solution (e.g. 1:50 dilution) in TE (10 mM Tris and 1 mM EDTA, pH 7.4). Measure absorbance of the diluted sample in a 1 mL cuvette using a traditional UV-VIS spectrophotometer at 260 and 280 nm. The spectrophotometer should first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

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

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 Agarose Gel Electrophoresis

The overall integrity and size distribution of total RNA purified with SurePrep Kits can be evaluated by denaturing agarose gel electrophoresis with ethidium bromide staining or by using the Agilent 2100 bioanalyzer. The respective ribosomal RNAs should appear as distinct bands in the gel or sharp peaks in the electropherogram. The intensity of the upper (28S) band should be about twice that of the lower (18S) band (size is dependent on the organism from which the RNA was obtained). It is common to see a diffuse smear of ethidium bromide staining between the sharp 18S and 28S ribosomal bands, probably consisting of various mRNA species. Small RNA species such as tRNA, 5S rRNA, and microRNA < 200 nucleotides in size will appear as a discernable band(s). If the ribosomal bands or peaks appear as a smear towards smaller sized RNAs, it is likely that the sample was degraded either before or during the purification process.



Total RNA isolated from *E. coli* in log-phase growth using the TrueTotal RNA Purification Kit. One µg of total RNA isolated from *E. coli* (strain FB5α, Fisher Cat. # BP4000-3) was loaded in lanes 2-4 on a 1.5% denaturing formaldehyde-agarose gel. Lane 1 contained a 1kb RNA standard (BP2811). Electrophoresis was performed at 100 V for 70 min and the gel stained with ethidium bromide.

F. 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.

IV. Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Incomplete lysis of cells or tissue	Ensure that the appropriate amount of Lysis Solution was used for the amount of cells or tissue.
	Column has become clogged	Do not exceed the recommended amounts of starting biological material. 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.
	Low RNA content in cells or tissues used	Different tissues and cells have different RNA contents, and thus the expected yield of RNA will vary greatly from these different sources. Please check literature to determine the expected RNA content of your starting material.
	Cell Culture: Cell monolayer was not washed with PBS	Ensure that the cell monolayer is washed with the appropriate amount of PBS in order to remove residual media from cells.
	Yeast: Lyticase was not added to the Resuspension Buffer	Ensure that the appropriate amount of lyticase is added when making the Resuspension Buffer.
	Bacteria and Yeast: All traces of media not removed	Ensure that all media is removed prior to the addition of the lysis solution through aspiration.
Clogged Column	Insufficient solubilization of cells or tissues	Ensure that the appropriate amount of lysis buffer was used for the amount of cells or tissue.
	Maximum number of cells or amount of tissue exceeds kit specifications	Refer to specifications to determine if amount of starting material falls within kit specifications
	Clarified lysate was not used for the binding step	Ensure that after the lysis step, the sample is centrifuged if any precipitates are present, and that only the clarified lysate is used in subsequent steps.
	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*" in Section I of this user guide.
	Cell lysis procedure not performed quickly enough	In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly. This is especially important for the Cell Lysate Preparation Step in the Animal Tissue protocol, since the RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized.

	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.
	Frozen tissues or cell pellets were allowed to thaw prior to RNA isolation	Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised.
	Starting material may have a high RNase content	For starting materials with high RNAase content, it is recommended that β-mercaptoethanol be added to the Lysis Solution.
	Lysozyme or lyticase used may not be RNAse-free	Ensure that the lysozyme and lyticase being used with this kit is RNase-free, in order to prevent possible problems with RNA degradation.
RNA does not perform well in downstream applications	RNA was not washed 3 times with the provided Wash Solution	Traces of salt from the binding step may remain in the sample if the column is not washed 3 times with 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.
Genomic DNA contamination	Large amounts of starting material used	Perform RNAse-free DNaseI digestion on the RNA sample after elution to remove genomic DNA contamination.

V. 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

VI. Appendix A

Protocol for Optional On-Column DNA Removal

TrueTotal RNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional protocol is provided below for maximum removal of residual DNA that may affect sensitive downstream applications. It is recommended that an RNase-free DNase I be used.

1. Prepare a working stock of 0.25 Kunitz unit/µL RNase-free DNase I solution according to the manufacturer's instructions. A 100 µL aliquot is required for each column to be treated. 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 0.25 Kunitz unit/µL.

2. Perform the appropriate Total RNA Isolation Procedure for your starting material up to and including "**Binding RNA to Column**" in Section IIIA.

3. Apply 400 µL of **Wash Solution** to the column and centrifuge for 2 minute. Discard the flow-through. Reassemble the spin column with its collection tube.

4. Apply 100 μL of the RNase-free DNase I solution prepared in Step 1 to the column. Centrifuge for 30 sec at 200 x g (~1,500 RPM). Alternatively, centrifuge for a 5 second pulse at 14, 000 x g (~12,000 RPM) if only a single speed centrifuge is available. Approximately half of the DNase I solution will pass through the column.

5. Incubate the column assembly at 25 - 30°C for 15 minutes.

6. Without any further centrifugation, proceed directly to "**Column Wash**" (Section IIIB).

Technical Support

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