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Fisher BioReagents®

SurePrep™ RNA/Protein Purification Kit

Product Cat. # BP2806-50

Instruction Manual

I. Introduction

A. Product Description

The SurePrep™ RNA/Protein Purification Kit provides a rapid method for the isolation and purification of total RNA and proteins sequentially from a single sample of cultured animal cells, tissue samples, blood, bacteria, yeast, fungi or plants. The total RNA and proteins are both column-purified in under 20 minutes using the same column. It is often necessary to isolate total RNA and proteins from a single sample, such as for studies of gene expression including gene silencing experiments, mRNA knockdowns or experiments correlating RNA and protein expression levels. Traditionally the RNA and proteins would be isolated from different aliquots of the same sample, however this novel technology will allow for their simultaneous isolation from the same sample. This will not only save time, but will also be of a great benefit when isolating RNA and proteins from precious, difficult to obtain or very small samples. Furthermore, gene expression analysis will be more reliable since the RNA and proteins are derived from the same sample, therefore eliminating inconsistent results.

B. Overview of Procedure

RNA Purification

Purification is based on spin column chromatography using a proprietary resin as the separation matrix. The process involves first lysing the cells or tissue of interest with the provided Lysis Solution (please see the flow chart on page 6). The Lysis Solution contains detergents, as well as large amounts of a chaotropic denaturant that will rapidly inactivate RNases and proteases that are present. Ethanol is then added to the lysate, and the solution is loaded onto a spin-column. Fisher's resin binds nucleic acids in a manner that depends on ionic concentrations, thus only the genomic DNA and RNA will bind to the column while the proteins are removed in the flowthrough. Next, an optional step can be carried out in which the genomic DNA can be digested allowing for a more pure RNA sample to be isolated. (Alternatively, the RNA may be digested at this point if a pure sample of genomic DNA is required instead). The bound RNA is then washed twice with the provided Wash Solution in order to remove any impurities, and the purified RNA is eluted with the Elution Buffer.

The kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). The purified RNA is of the highest quality, and can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection, and expression array assays.

Protein Purification

The proteins that are present from the initial flowthrough can now be loaded directly onto an SDS-PAGE gel for visual analysis. Alternatively, the protein samples can be further purified using the

spin columns provided with the kit. After the desired nucleic acids have been eluted from the column, the column can be regenerated using the supplied Regeneration Buffer, followed by column activation using the supplied Activation and Wash Buffer. The flowthrough is then pH adjusted and loaded onto the regenerated column in order to bind the proteins that are present. The bound proteins are washed with the provided wash buffer, and are then eluted such that they can be used in downstream applications. The purified proteins can be used in a number of downstream applications including SDS-PAGE analysis or Western blots.

C. Kit Specifications

Kit Specifications	
Column Binding Capacity	50 μg for RNA 200 μg for protein
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 10 Purifications	30 minutes
Average Yields* HeLa Cells (1×10^6 cells) HeLa Cells (1×10^6 cells)	 15 μg RNA 150 μg protein

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

D. Advantages

- Fast and easy processing using rapid spin-column format
- All columns for RNA purification and protein purification provided
- Sequentially isolate nucleic acids and proteins from a single lysate – no need to split the lysate
- Isolate all sizes of RNA, from large mRNA and rRNA down to microRNA (miRNA)
- No phenol or chloroform extractions
- Isolate high quality total RNA
- High yields of isolated proteins

E. Kit Components

Component	Catalog # BP2806-50 (50 preps)
Lysis Solution	40 mL
Nucleic Acid Wash Solution	22 mL
Nucleic Acid Elution Buffer	15 mL
Protein Column Regeneration Buffer	30 mL
Protein Column Activation and Wash Buffer	60 mL
Protein pH Binding Buffer	4 mL
Protein Elution Buffer	8 mL
Enzyme Incubation Buffer	6 mL
Protein Neutralizer	2 mL
Protein Loading Dye	2 mL
Spin Columns	50
Collection Tubes	150
Product Insert	1

F. Storage Conditions and Product Stability

The Protein Loading Dye should be stored at -20°C upon arrival. All other solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year 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 is 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 SurePrep™ RNA/Protein Purification Kit:

For All Protocols

- Benchtop microcentrifuge
- β-mercaptoethanol (Optional)
- 95% ethanol
- RNase-free DNase I (Optional)
- Isopropanol
- Molecular biology grade water (e.g. BP561 Sterile Water for RNA work)

- RNase/DNase-Free Microcentrifuge tubes

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

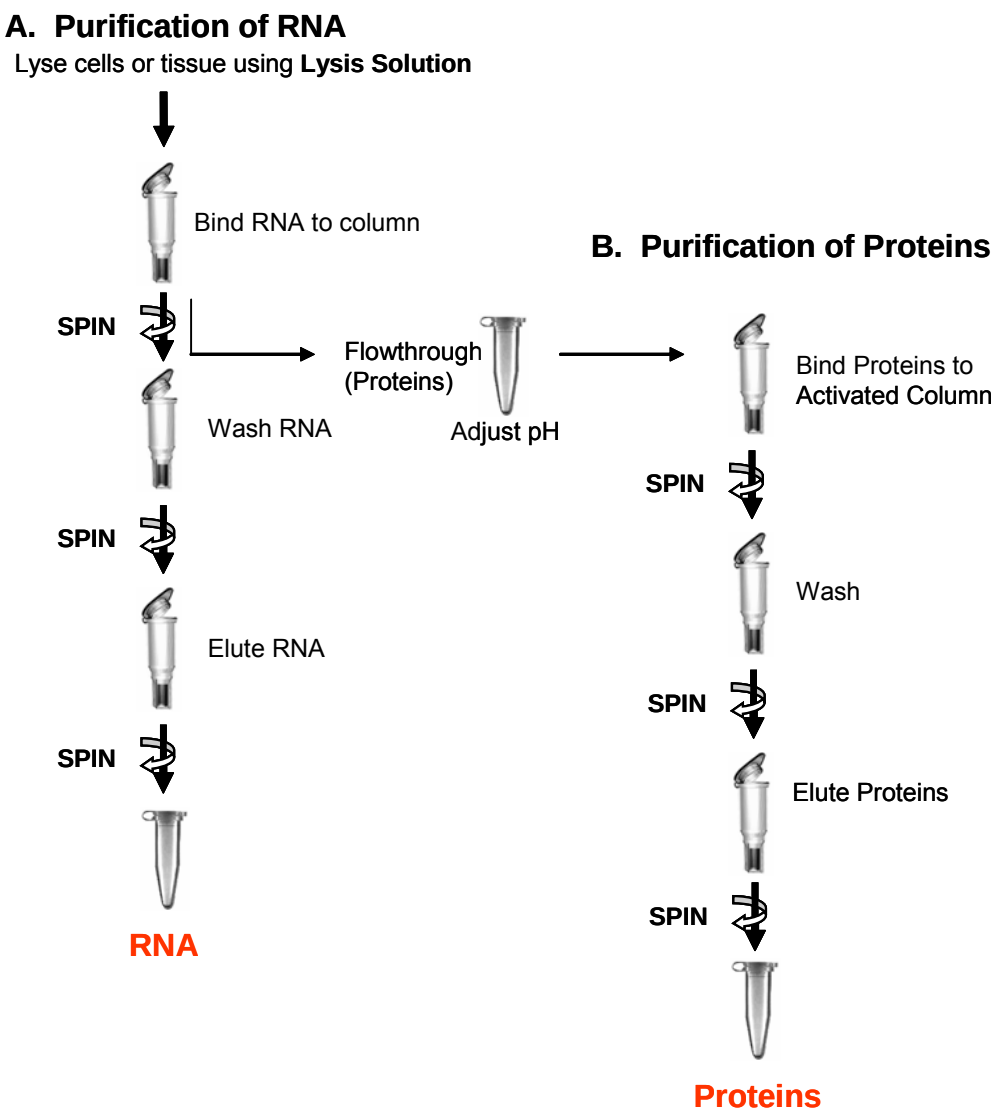
I. Working with RNA

RNases are 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 Total RNA and Proteins using SurePrep™ RNA/Protein Purification Kit



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**

IMPORTANT NOTE:

This procedure is written in three steps. The first step is to prepare the cell lysate. Please ensure that the proper protocol is followed for your particular biological sample. Step 2 contains the protocol to purify total RNA from the different cell lysates. Step 3 includes the protocol to isolate total proteins from the same sample (see flow chart on page 6).

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 to I). However, the subsequent steps for purifying total RNA and total proteins are the same for all types of cell lysate (Sections III and IV).
- 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 **Nucleic Acid Wash Solution** by adding 50 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated **RNA 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 when isolating RNA.
- 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

- For optimal results, it is recommended that 1 x 10^6 cells be used for the input. However, inputs of up to 3 x 10^6 cells may be used.
- 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 150 µL of isopropanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds. **Proceed to Section III.**

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 reduce the viscosity of the lysate prior to loading onto the column.

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 to ensure that the pellet is not dislodged. Wash the cell pellet with an appropriate amount of PBS. Centrifuge at 200 x g (~1,500 RPM) for another 5 minutes.
c. Carefully decant the supernatant. A few microliters of PBS may be left behind with the pellet in order to ensure that the pellet is not dislodged.
d. 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.
e. Add 150 µL of isopropanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds. **Proceed to Section III.**

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 reduce the viscosity of the lysate prior to loading onto the column.

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. 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.
- For optimal results, it is recommended that no more than 25 mg of tissue be processed.

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. Centrifuge at 12,000 RPM for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube (not provided). Note the volume of the supernatant/lysate.
h. Add an equal volume of 70% ethanol (provided by the user) to the lysate (100 μL of ethanol is added to every 100 μL of lysate). Mix by vortexing for 10 seconds. **Proceed to Section III.**

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 150 μL of isopropanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds. **Proceed to Section III.**

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.
d. Add 300 μL of Lysis Solution and vortex vigorously for at least 10 seconds.
e. Add 150 μL of 95% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds. **Proceed to Section III.**

Table 1: Incubation Time for Different Bacterial Strains

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

G. Lysate Preparation from Yeast

Notes Prior to Use

- Prepare the appropriate amount of Lyticase-containing Resuspension Buffer, considering that 200 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 from Yeast

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 500 μL of Lyticase-containing Resuspension Buffer by vortexing. Incubate at 37°C for 10 minutes.
d. Pellet spheroplast at 200 *x g* (~1,500 RPM) for 3 minutes. Decant supernatant.
e. Add 350 μL of Lysis Solution and vortex vigorously for at least 10 seconds.
f. Add 150 μL of isopropanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds. **Proceed to Section III.**

H. Lysate Preparation from Fungi

Notes Prior to Use

- Fresh or frozen fungi may be used for this procedure. Fungal tissue 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 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 equal volume of 70% ethanol (provided by the user, 100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix. **Proceed to Section III.**

I. Lysate Preparation from Plant

Notes Prior to Use

- The maximum recommended input of plant tissue is 50 mg or 5×10^6 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 5×10^6 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 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 equal volume of 70% ethanol (provided by the user, 100 μL of ethanol is added to every 100 μL of lysate). Vortex to mix. **Proceed to Section III.**

III. Total RNA Purification from All Types of Sample Lysate

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

A. Binding RNA to Column

a. Assemble a spin column with one of the provided collection tubes.
b. Apply up to 600 μL of the clarified lysate with the ethanol (or isopropanol) onto the column and centrifuge for 1 minute.

Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute.

c. **Retain the flowthrough for Protein Purification (Section IV). The flowthough contains the proteins and should be stored on ice or at -20°C until the Protein Purification protocol is carried out.**
d. Depending on your lysate volume, repeat steps **b** and **c**, if necessary. The flowthroughs should be combined and retained in the same microcentrifuge tube.
e. Reassemble the spin column with a new collection tube.

Note: If RNA is required that is completely free of genomic DNA, proceed to step **III D** for an optional DNase treatment.

B. Column Wash

a. Apply 400 μL of **Nucleic Acid 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 column with the collection tube.
c. Wash column a second time by adding another 400 μL of **Nucleic Acid Wash Solution** and centrifuge for 2 minutes.
d. Ensure that the column is dry. Spin for an additional minute, if necessary.
e. Discard the collection tube with the flowthrough.

C. RNA Elution

a. Place the column into a fresh microcentrifuge tube (not provided).
b. Add 50 µL of **Nucleic Acid 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 volume has not been eluted, spin the column at 14,000 x g (~12,000 RPM) for 1 additional minute.

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

d. **Retain the column for Protein Purification**. Proceed to Section IV for Protein Purification.

D. DNase Treatment (Optional)

This optional step can be carried out if RNA completely free of genomic DNA is required.

a. After binding of RNA to column (step **A**), apply 400 µL of **Nucleic Acid Wash Solution** to the column and centrifuge for 2 minutes. Discard the flowthrough.

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. Apply 100 µL of **Enzyme Incubation Buffer** containing 25 units of RNase-free DNase I (user provided) to the column.
c. Centrifuge for 30 seconds at **200 x *g* (~1,500 RPM)** to allow half of the **Enzyme Incubation Buffer** containing DNase I to pass through the column. Alternatively, if the centrifuge used has no speed adjustment, centrifuge for 5 seconds at 14000 x g (~12000 RPM).
d. Incubate the whole unit at room temperature for 15 minutes.
e. Proceed to step **C** (Column Wash) without further centrifugation.

E. 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.

F. 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.

G. 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 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.

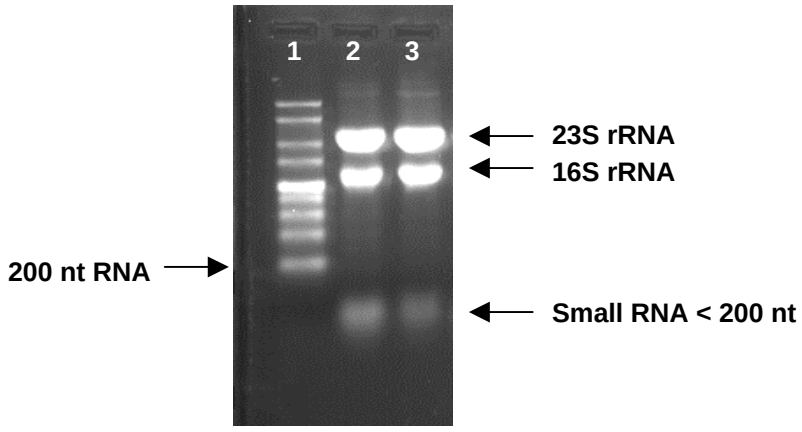


Fig. 1. Total RNA isolated from *E. coli* in log-phase growth using the SurePrep™ RNA/Protein Purification Kit.
One µg of total RNA isolated from *E. coli* (strain FB5α, Fisher Cat. # BP4000-3) was loaded in lanes 2 and 3 on a 1.5% denaturing formaldehyde-agarose gel. Lane 1 contained a 1kb RNA standard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

IV. Procedure to Isolate Total Proteins from All Cell Types

Notes Prior to Use

- At this point, the proteins that are present in the flowthrough from the RNA Binding Step (Section III) can be loaded directly onto an SDS-PAGE gel for visual analysis, or the proteins can be further purified using the protocol below.
- For direct running on a gel, the provided **Protein Loading Dye** should be used instead of regular SDS-PAGE Loading Buffer in order to prevent any precipitates from forming. Add 1 volume of the **Protein Loading Dye** to the sample and boil for 2 minutes before loading.

A. Column Regeneration

a. After RNA Elution, retain the spin column and assemble it with one of the provided collection tubes.
b. Add 500 μL of **Protein Column Regeneration Buffer** to the column and centrifuge at 14,000 x g (12,000 RPM) for 2 minutes.
c. Discard the flowthrough. Reassemble the spin column with its collection tube.

B. Column Activation for Protein Binding

a. Add 500 μL of **Protein Column Activation and Wash Buffer** to the column and centrifuge at 14,000 x g (12,000 RPM) for 2 minutes.
b. Discard the flowthrough. Reassemble the spin column with its collection tube.

C. pH Adjustment of Lysate

a. Transfer 100 μL of flowthrough from the RNA Binding Step (Section III) to a separate microcentrifuge tube.

Note: Up to 300 μL of flowthrough may be used. However, the recovery efficiency may be decreased when processing a larger volume.

b. Adjust volume to 600 μL with molecular biology grade water.
c. Add 24 μL of **Protein pH Binding Buffer**. Mix contents well.
d. Verify that the pH is 3.5 or lower, and add more pH Binding Buffer if necessary.

Note: If the entire lysate is to be purified, repeat steps **a** to **d** with the remaining lysate.

D. Protein Binding

a. Apply the entire pH-adjusted protein sample onto the column, and centrifuge for two minutes at 5,200 x g (~8,000 RPM). Inspect the column to ensure that the entire sample has passed through into the collection tube. If necessary, spin for an additional three minutes.

Note: If the volume of pH-adjusted protein sample exceeds the capacity of the column (650 μL), divide the sample into two spins.

b. Discard the flowthrough. Reassemble the spin column with its collection tube.

Note: You can save the flowthrough in a fresh tube for assessing your protein's binding efficiency.

E. Column Wash

a. Apply 500 μL of **Protein Column Activation and Wash Buffer** to the column and centrifuge for two minutes at 5,200 x g (8000 RPM).
b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Inspect the column to ensure that the liquid has passed through into the collection tube. There should be no liquid in the column. If necessary, spin for an additional minute to dry.

F. Protein Elution and pH Adjustment

Note: The supplied **Protein Elution Buffer** consists of 10 mM sodium phosphate pH 12.5.

a. Add 9.3 μL of **Protein Neutralizer** to a microcentrifuge tube (not provided).
b. Transfer the spin column from the Column Wash procedure into the microcentrifuge tube.
c. Apply 100 μL of the **Protein Elution Buffer** to the column and centrifuge for two minutes at 5,200 x g (8000 RPM) to elute bound proteins.

Note: Approximately 95% of bound protein is recovered in the first elution. If desired, a second elution using 50 μL of **Protein Elution Buffer** may be carried out. This should be collected into a different tube (to which 4.6 μL of **Protein Neutralizer** is pre-added) to prevent dilution of the first elution.

G. Storage of Purified Protein

It is recommended that samples be placed at –20°C for long term storage.

H. Assessing Protein Quality by SDS-PAGE

The general quality of the total protein isolated by the SurePrep Kit can be assessed with SDS-PAGE. Select a suitable procedure to separate the molecular mass range of the proteins of interest. For example, use a gradient gel if a wide range of protein molecular masses needs to be covered. Ten μL of each 100 μL elution is typically run on the gel. Alternatively, the proteins from the initial lysate may also be directly loaded onto an SDS-PAGE gel without column purification using the provided Protein Loading Dye.

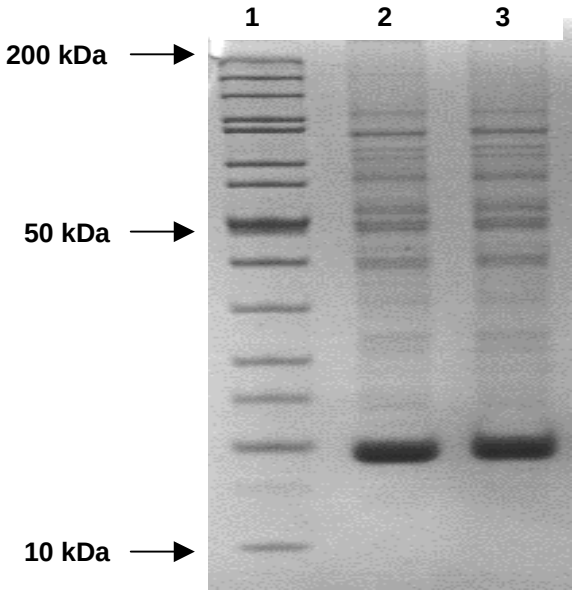


Fig. 2. Total protein isolated from *E. coli* in log-phase growth using the SurePrep™ RNA/Protein Purification Kit.
Total protein isolated from the same *E. coli* cell samples used in Figure 1. Ten μL of the 100 μL protein elution was loaded in lanes 2 and 3 on a 12.5% EZ-Run™ Protein Gel. Lane 1 contains Fisher's EZ-Run *Rec* Protein Ladder (BP3602). Electrophoresis was performed at 150 V for 90 minutes and the gel stained with EZ-Run Protein Gel Staining Solution (BP3620-1).

V. 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 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 RNA elution solution was used	It is recommended that the **Nucleic Acid Elution Buffer** supplied with this kit be used for maximum RNA recovery.
	Alcohol was not added to the lysate	Ensure that the appropriate amount of isopropanol or ethanol is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution	Ensure that 50 mL of 100% ethanol is added to the supplied **Nucleic Acid 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 the sample is centrifuged after the lysis step if any cell debris is present, and only the clarified lysate is used in subsequent steps.

Problem	Possible Cause	Solution and Explanation
Clogged 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. 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.
	DNase I used may not be RNase-free	Ensure that the DNase I being used with this kit is RNase-free in order to prevent possible problem with RNA degradation.
	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.
	Starting material may have a high RNase content	For starting materials with high RNase content, it is recommended that β-mercaptoethanol be added to the Lysis Solution.
	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.
RNA does not perform well in downstream applications	RNA was not washed twice with the provided Wash Solution	Traces of salt from the binding step may remain in the sample if the column is not washed twice with **Nucleic Acid 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.

Problem	Possible Cause	Solution and Explanation
Poor protein recovery	Incorrect pH adjustment of sample.	Ensure that the pH of the starting protein sample is adjusted to pH 3.5 using the provided **Protein pH Binding Buffer**
	Low protein content in the starting materials	Run a 20 µL fraction from the flowthrough (after Nucleic Acid binding) on a SDS-PAGE gel to estimate the amount of protein present in the sample. In addition, use the entire flowthrough in protein purification procedure.
Proteins are degraded	Eluted protein solution was not neutralized.	Add 9.3 µL of **Protein Neutralizer** to each 100 µL of eluted protein in order to adjust the pH to neutral. Some proteins are sensitive to high pH, such as the elution buffer at pH 12.5.
	Eluted protein was not neutralized quickly enough.	If eluted proteins are not used immediately, degradation will occur. We strongly suggest adding **Protein Neutralizer** in order to lower the pH.

VI. 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, DNA, and Protein 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
BP1333-1	Tris-Borate-EDTA 10X Solution 1L
BP1335-1	Tris-Acetate-EDTA 10X Solution 1L
BP308-100	MOPS Buffer 100g
BP2580-100	exACTGene 24kb Max DNA Ladder 100 loadings
BP3602-500	EZ-Run™ *Rec* Protein Ladder 100 loadings
BP7712-50	EZ-Run™ 12.5% Protein Gel Solution 50 mini-gels
BP7700-500	EZ-Run™ Running Buffer 20X 500ml
BP3620-1	EZ-Run™ Protein Gel Staining Solution

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