Fisher BioReagents®

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SurePrep™ Nuclear or Cytoplasmic RNA Purification Kit

Product Cat. # BP2805-25

Instruction Manual

I. Introduction

A. Product Description

The SurePrep™ Nuclear or Cytoplasmic RNA Purification Kit provides a rapid method for the isolation and purification of both nuclear and cytoplasmic RNA from cultured animal cells and small tissue samples. In certain circumstances it is desirable to be able to isolate fractionated RNA as opposed to total RNA. For example, it may be preferable to isolate only mature, cytoplasmic RNA for some studies on expression profiling. Alternatively, it may be desirable to isolate nuclear RNA in order to investigate and study pre-processed (non-spliced) RNA. Furthermore, this kit can be used to isolate RNA for downstream applications where it is necessary to avoid DNA contamination, since the cytoplasmic fraction has been shown to be free of all traces of genomic DNA. Fisher's Nuclear or Cytoplasmic RNA Purification Kit can be used to isolate all sizes of RNA from the cytoplasmic and nuclear RNA fractions, including all small RNA species. The kit is supplied with sufficient reagents to perform either 50 cytoplasmic RNA preparations or 25 cytoplasmic and nuclear RNA preparations.

B. Overview of Procedure

Purification is based on spin column chromatography using a proprietary resin as the separation matrix. The cytoplasmic RNA is preferentially purified from the nuclear RNA and other cellular components such as proteins without the use of phenol or chloroform. The process involves first lysing the cells or tissue of interest with the provided Lysis Solution (please see the flow chart on page 5). The lysate is then separated through centrifugation, with the supernatant containing the cytoplasmic RNA and the pellet containing the nuclear RNA. Binding solution and ethanol is then added to the desired fraction, and the solution is loaded onto a spin-column. Fisher's resin binds RNA in a manner that depends on ionic concentrations. Thus, only the RNA will bind to the column while the contaminating proteins will be removed in the flowthrough or retained on the top of the resin. The bound RNA is then washed with the provided wash solution in order to remove any remaining impurities, and the purified RNA is eluted with the elution buffer. 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 primer extension, and expression array assays.

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	 3×10^6 cells 15 mg
Time to Complete 10 Purifications	45 minutes
Average Yields* HeLa Cells – cytoplasmic (1×10^6 cells) HeLa Cells – nuclear (1×10^6 cells)	 15 μg $\leq$ 3.5 μg

***** *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
- Isolate fractionated RNA from a variety of sources
- No phenol or chloroform extractions
- No genomic DNA contamination in the cytoplasmic fraction
- Isolate all sizes of RNA from each fraction, including all small RNA molecules (<200 nt)
- High quality RNA can be used in various downstream applications

E. Kit Components

Component	Catalog # BP2805-25
Lysis Solution	20 mL
Binding Solution	25 mL
Wash Solution	22 mL
RNA Elution Solution	6 mL
Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

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.

H. Customer-Supplied Reagents and Equipment

You must have the following in order to use the SurePrep™ Nuclear or Cytoplasmic RNA Purification Kit

For All Protocols

- Benchtop microcentrifuge
- β-mercaptoethanol
- 95 - 100% ethanol

For Animal Tissue Protocol

- Homogenizer (e.g. mortar and pestle, microtube pestle)

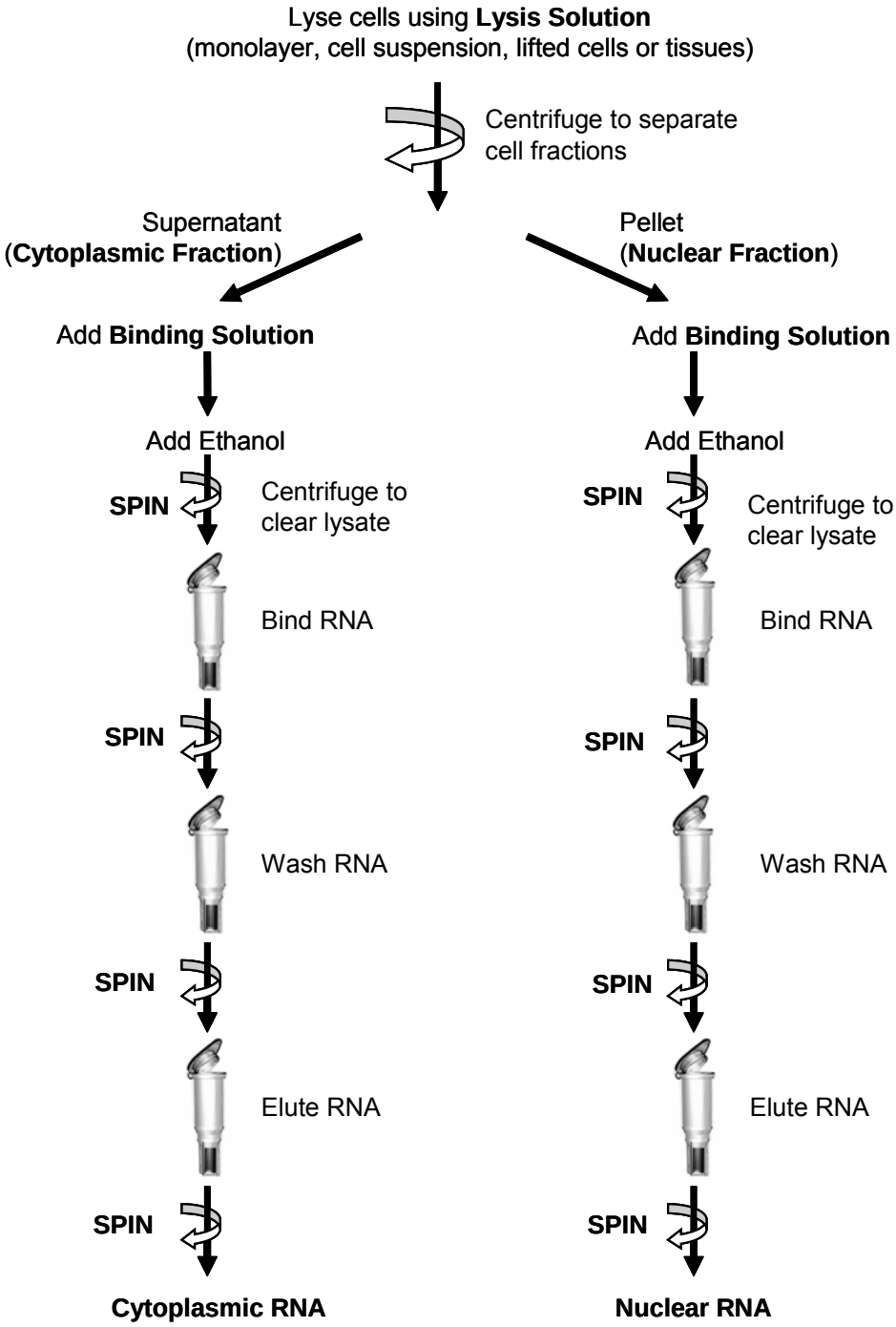
I. Working with RNA

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 Nuclear and Cytoplasmic RNA Molecules



II. Purification of Cytoplasmic and Nuclear RNA from Biological Samples

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 specifications on the Sorvall Legend MicroCentrifuges please visit www.thermo.com.

B. Protocols for Purification of RNA from Cultured Animal Cells

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 except **Lysis Solution** are at room temperature prior to use.
- Prepare an appropriate amount of **Lysis Solution** by adding 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of **Lysis Solution** required. Place the solution on ice or at 4°C prior to use. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
- 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.
- 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.
- The maximum recommended amount of starting material is 3 x 10^6 cells. 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.
- Fresh cultured animal cells are the recommended input for this procedure.
- 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.
- It is important to work quickly during this procedure.

Cells Growing in a Monolayer

1. Cell Fraction Preparation

a. Aspirate media and wash cell monolayer with an appropriate amount of PBS. Aspirate PBS.
b. Add 200 µL of ice-cold **Lysis Solution** directly to culture plate.
c. Lyse cells by gently tapping culture dish and swirling buffer around plate surface for five minutes, while keeping the cell culture plate on ice.
d. Using a pipette, transfer lysate to an RNase-free microcentrifuge tube (not provided). Spin lysate for 3 minutes at maximum speed in a benchtop centrifuge. Transfer the supernatant containing **cytoplasmic RNA** to another RNase-free tube (not provided). If nuclear RNA extraction is desired, retain the pellet containing the **nuclear RNA**. Continue immediately to step 2.

Note: Depending on the amount of cells used, the nuclear RNA fraction (pellet) may or may not be visible. A few µL of **Lysis Solution** may be left behind with the pellet in order to ensure that the pellet is not dislodged.

2A. Binding Cytoplasmic RNA to Column

a. Add 200 µL of **Binding Solution** to the supernatant **(cytoplasmic RNA fraction)** from step **1d.** Mix by vortexing for 10 seconds.

b. Add 200 µL of 95 - 100% ethanol (provided by the user) to the mixture**.** Mix by vortexing for 10 seconds.
c. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to step d without centrifugation.
d. Apply the clarified fraction with the ethanol onto a spin column that has been assembled with a collection tube and centrifuge for 1 minute.
e. Discard the flowthrough. Reassemble the spin column with its collection tube.

2B. Binding Nuclear RNA to Column

a. Add 400 µL of **Binding Solution** to the pellet **(nuclear RNA fraction)** from step **1d**. Mix by vortexing for 10 seconds.
b. Add 200 µL of 95 - 100% ethanol (provided by the user) to the mixture**.** 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.

c. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to step d without centrifugation.
d. Apply the clarified fraction with the ethanol onto a spin column that has been assembled with a collection tube and centrifuge for 1 minute.
e. Discard the flowthrough. Reassemble the spin column with its collection tube.

Optional Step:
The SurePrep™ Nuclear or Cytoplasmic RNA Purification Kit isolates nuclear 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 applications. This step should be performed at this point in the protocol. The DNA Removal Protocol is not required for downstream applications of the cytoplasmic fraction.

3. Column Wash

a. Apply 400 µL of **Wash Solution** to the column and centrifuge for 1 minute. 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. Repeat step **3a** to wash column a second time.
c. Wash column a third time by adding another 400 µL of **Wash Solution** and centrifuging 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.

4. RNA Elution

a. Place the column into a fresh 1.7 mL elution tube provided with the kit.
b. Add 50 µL of **RNA Elution Solution** 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 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.

Cells Growing in Suspension and Lifted Cells

1. Cell Fraction Preparation

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 microliters of media may be left behind with the pellet in order to ensure that the pellet is not dislodged.
c. Add 200 µL of ice-cold **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. Spin lysate for 3 minutes at maximum speed in a benchtop centrifuge. Transfer the supernatant containing **cytoplasmic RNA** to an RNase-free tube (not provided). If nuclear RNA extraction is desired, retain the pellet containing the **nuclear RNA**. Continue immediately to step **2**.

Note: Depending on the amount of cells used, the nuclear RNA fraction (pellet) may or may not be visible. A few microliters of **Lysis Solution** may be left behind with the pellet in order to ensure that the pellet is not dislodged.

2A. Binding Cytoplasmic RNA to Column

a. Add 200 µL of **Binding Solution** to the supernatant **(cytoplasmic RNA fraction)** from step **1d.** Mix by vortexing for 10 seconds.
b. Add 200 µL of 95 - 100% ethanol (provided by the user) to the mixture**.** Mix by vortexing for 10 seconds.
c. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to step **d** without centrifugation.
d. Apply the clarified fraction with the ethanol onto a spin column that has been assembled with a collection tube, and centrifuge for 1 minute.
e. Discard the flowthrough. Reassemble the spin column with its collection tube.

2B. Binding Nuclear RNA to Column

a. Add 400 µL of **Binding Solution** to the pellet **(nuclear RNA fraction)** from step **1d**. Mix by vortexing for 10 seconds.
b. Add 200 µL of 95 - 100% ethanol (provided by the user) to the mixture**.** 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.

c. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to step **d** without centrifugation.
d. Apply the clarified fraction with the ethanol onto a spin column that has been assembled with a collection tube, and centrifuge for 1 minute.
e. Discard the flowthrough. Reassemble the spin column with its collection tube.

Optional Step:
The SurePrep™ Nuclear or Cytoplasmic RNA Purification Kit isolates nuclear 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 applications. This step should be performed at this point in the protocol. The DNA Removal Protocol is not required for downstream applications of the cytoplasmic fraction.

3. Column Wash

a. Apply 400 µL of **Wash Solution** to the column and centrifuge for 1 minute. 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. Repeat step **3a** to wash column a second time.
c. Wash column a third time by adding another 400 µL of **Wash Solution** and centrifuging 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.

4. RNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 50 µL of **RNA Elution Solution** 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 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 Purification of RNA from Animal Tissue

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 except **Lysis Solution** are at room temperature prior to use.
- Prepare an appropriate amount of **Lysis Solution** by adding 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of **Lysis Solution** required. Place the solution on ice or at 4°C prior to use. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
- 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.
- 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.
- 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 Fraction Preparation step.
- Fresh tissues are highly recommended for this procedure.
- Frozen tissues may be used for this 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 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 15 mg of tissue be used in order to prevent clogging of the column.

1. Cell Fraction Preparation

a. Excise the tissue sample from the animal.
b. Determine the amount of tissue by weighing. It is recommended that no more than 15 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 200 µL of **Lysis Solution** to the tissue sample and continue the homogenization until the tissue dissolves.
f. Using a pipette, transfer lysate to an RNase-free microcentrifuge tube (not provided). Spin lysate for 3 minutes at maximum speed in a benchtop centrifuge. Transfer the supernatant containing **cytoplasmic RNA** to another RNase-free tube (not provided). If nuclear RNA extraction is desired, retain the pellet containing the **nuclear RNA**. Continue immediately to step **2**.

Note: Depending on the type of tissue used and the extent of homogenization, significant **cytoplasmic RNA** contamination in the **nuclear RNA** fraction may occur.

2A. Binding Cytoplasmic RNA to Column

a. Add 200 µL of **Binding Solution** to the supernatant **(cytoplasmic RNA fraction)** from step **1f.** Mix by vortexing for 10 seconds.
b. Add 200 µL of 95 - 100% ethanol (provided by the user) to the mixture**.** Mix by vortexing for 10 seconds.
c. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to step **d** without centrifugation.

d. Apply the clarified fraction with the ethanol onto a spin column that has been assembled with a collection tube, and centrifuge for 1 minute.
e. Discard the flowthrough. Reassemble the spin column with its collection tube.

2B. Binding Nuclear RNA to Column

a. Add 400 µL of **Binding Solution** to the pellet **(nuclear RNA fraction)** from step **1f**. Mix by vortexing for 10 seconds.

b. Add 200 µL of 95 - 100% ethanol (provided by the user) to the mixture**.** Mix by vortexing for 10 seconds.

Note: For input amounts greater than 10 mg, 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.

c. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to step **d** without centrifugation.
d. Apply the clarified fraction with the ethanol onto a spin column that has been assembled with a collection tube, and centrifuge for 1 minute.
e. Discard the flowthrough. Reassemble the spin column with its collection tube.

Optional Step:

The SurePrep™ Nuclear or Cytoplasmic RNA Purification Kit isolates nuclear 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 applications. This step should be performed at this point in the protocol. The DNA Removal Protocol is not required for downstream applications of the cytoplasmic fraction.

3. Column Wash

a. Apply 400 µL of **Wash Solution** to the column and centrifuge for 1 minute. 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. Repeat step **3a** to wash column a second time.
c. Wash column a third time by adding another 400 µL of **Wash Solution** and centrifuging 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.

4. RNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 50 µL of **RNA Elution Solution** 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 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.

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 appropriate aliquot of the purified RNA solution 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.

III. Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Insufficient solubilization 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 buffer was used	It is recommended that the **RNA Elution Solution** 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.
	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.
Clogged Column	Insufficient solubilization of cells or tissues	Ensure that the appropriate amount of **Lysis Solution** 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 prior to the column binding step (step **d** in 2A and 2B), the sample is centrifuged for at least 1 minute and only the clarified lysate is used in subsequent steps.
	High amounts of genomic DNA present in sample	The nuclear lysate fraction 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.

Problem	Possible Cause	Solution and Explanation
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 the **Working with RNA** section 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.
	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.
	Tissue samples were frozen improperly	Samples should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage.
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 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 in cytoplasmic fraction	Traces of nuclear pellet remained in cytoplasmic fraction	Ensure that a solid pellet is formed at the end of the Cell Fraction Preparation step, and that none of the pellet is removed when the supernatant is transferred to another tube.

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
BP308-100	MOPS 100g
BP308-500	MOPS 500g

V. Appendix A

Protocol for Optional On-Column DNA Removal

The SurePrep™ Nuclear or Cytoplasmic RNA Purification Kit isolates nuclear 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 Nuclear RNA Isolation protocol for your starting material up to and including "**Binding Nuclear RNA to Column**" (step **2B** in all protocols).

3. Apply 400 μL of **Wash Solution** to the column and centrifuge for 2 minutes. Discard the flowthrough. 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 seconds 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 one 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 further centrifugation, proceed directly to "**Column Wash**" (step 3 of all protocols).

Technical Support

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