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Fisher BioReagents®

SurePrep™ Plant/Fungi Total RNA Purification Kit

Product Cat. # BP2817-50

Instruction Manual

I. Introduction

A. Product Description

Gene expression analysis using RNA has played a central role in the progressive understanding of many aspects of plant biology. The quality of extracted RNA is directly correlated to the success of various assays used to study gene expression such as RT-PCR and Northern blotting. The SurePrep™ Plant/Fungi Total RNA Purification Kit provides a rapid method for the isolation and purification of total RNA from a wide variety of plant and filamentous fungal species. Total RNA can be purified from fresh or frozen plant tissues, plant cells or calluses, hard fibrous tissues (seed, needles), or filamentous fungi samples using this kit. All sizes of RNA are purified, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). The procedure is rapid and convenient, as it does not rely on the use of liquid nitrogen in order to homogenize the samples. The RNA is preferentially purified from other cellular components, such as polysaccharides and proteins, without the use of phenol or chloroform. The purified RNA is of the highest quality, and can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays.

B. Overview of Procedure

Purification is based on spin column chromatography using a proprietary resin as the separation matrix. The RNA is preferentially purified from most other cellular macromolecules such as proteins and various secondary metabolites without the use of phenol or chloroform. The process involves first macerating the cells or tissue in a mortar with the provided Lysis Solution (flow chart on page 5). The Lysis Solution contains detergents, as well as large amounts of a chaotropic denaturant that will rapidly inactivate RNases and proteases that are present. Alternatively, liquid nitrogen can be used to homogenize the sample. The lysate is then spun in a microcentrifuge in order to pellet and remove any cellular debris. Ethanol is then added to the clarified 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 RNA will bind to the column while most of the DNA and proteins are removed in the flow-through. The bound RNA is then washed with the provided Wash Solution in order to remove any remaining impurities, and the purified total RNA is eluted with the Elution Buffer. The purified RNA is of the highest integrity, and can be used in a number of downstream applications.

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: Plant Tissues Plant Cells Fungi	 50 mg 1×10^6 cells 50 mg (wet weight)
Average Yields* 50 mg Tomato Leaves 50 mg Tobacco Leaves 50 mg Plum Leaves 50 mg Grape Leaves 50 mg Peach Leaves	 60 μg 60 μg 32 μg 35 μg 30 μg
Time to Complete 10 Purifications	30 minutes

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

D. Advantages

- Fast and easy processing using a rapid spin-column format
- Isolate the full complement of RNA, from large rRNA down to microRNA (miRNA)
- Liquid nitrogen is not required for tissue homogenization
- No phenol or chloroform extractions
- Common inhibitors of extracted plant RNA such as polyphenols and polysaccharides are effectively removed
- High yields of total RNA from a variety of plant and fungal species

E. Kit Components

Component	Catalog # BP2817-50 (50 preps)
Lysis Solution	40 mL
Wash Solution	22 mL
RNA Elution Buffer	6 mL
Spin Columns	50
Collection Tubes	50
Elution Tubes (1.7 mL)	50
Product Insert	1

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 Department.

H. Customer-Supplied Reagents and Equipment

You must have the following in order to use the SurePrep Plant/Fungi Total RNA Purification Kit:

- Benchtop microcentrifuge
- Mortar and pestle
- 95 - 100% ethanol
- β-mercaptoethanol
- 70% ethanol
- RNase-free DNase I (optional)
- Liquid nitrogen (optional)

I. Working with RNA

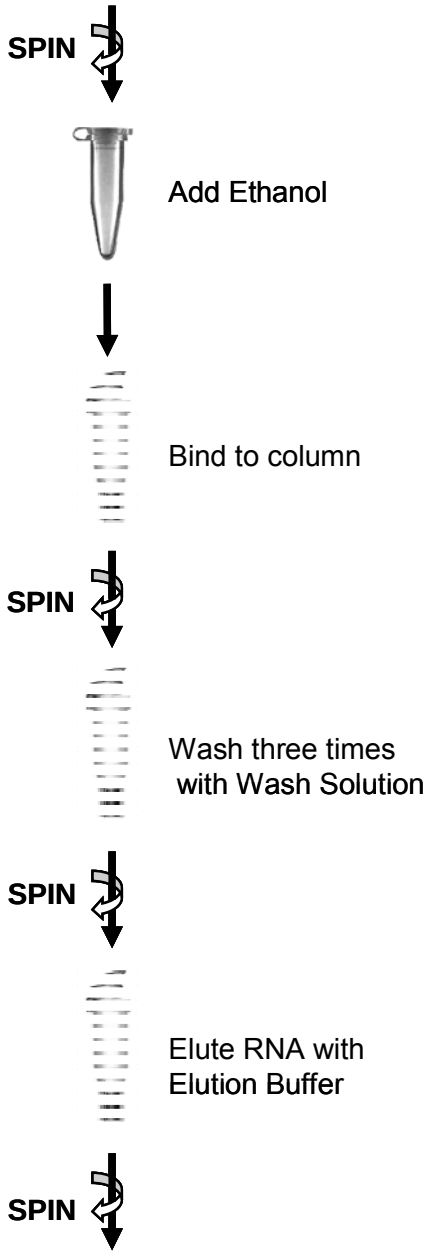
RNases are stable, robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to inactivate these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these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

Macerate cells or tissue in a mortar using **Lysis Solution**



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 Plant Cells or Tissue

Notes Prior to Use

- 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.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- 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 – 100 % 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.
- 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.
- Both fresh or frozen samples may be used for this procedure. Samples should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Do not allow frozen samples to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised.
- While the provided procedure does not rely on the use of liquid nitrogen to homogenize the sample, both fresh and frozen tissues can optionally be processed using other homogenization methods, including grinding with liquid nitrogen. Please refer to the Note below in **step a**.
- It is recommended that no more than 50 mg of fungi, 50 mg of plant tissue, or 5 x 10^6 plant cells be used for this procedure in order to prevent clogging of the column. However, in some cases it may be possible to increase the amount of plant material processed up to 100 mg or 5 x 10^7 cells, depending on the RNA content of the plant.
- 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.

Cell Lysate Preparation

a. Transfer ≤50 mg of plant tissue or 5 x 10^6 plant cells into a mortar that contains 600 µL of **Lysis Solution**. Grind the sample using a pestle until the tissue is completely macerated.

   Note: Other homogenization methods, including grinding with liquid nitrogen, can be applied to this procedure. If an alternative method is used, add 600 µL of **Lysis Solution** to the sample immediately after homogenization and vortex for 20 seconds to mix.

b. Using a pipette, transfer the lysate into an RNAase-free microcentrifuge tube (not provided).

c. 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.

   Note: Ensure that only the clear supernatant is transferred, avoiding any of the debris. If necessary, repeat **step c** if visible precipitates are still present after the first spin.

d. Add an equal volume of 70% ethanol (Molecular Biology Grade Ethanol, provided by the user) to the lysate collected above (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix. Proceed to **Section III A** Binding RNA to Column.

III. Purifying Total RNA from Sample Lysates

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 B**) onto the column and centrifuge for 1 minute at **14,000 x g (~12,000 RPM)**.
c. Discard the flowthrough. Reassemble the spin column with its collection tube.

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

d. Depending on your lysate volume, repeat **steps b** and **c** above.

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 1 minute.
e. Discard the flowthrough and reassemble the spin column with its collection tube.
f. Spin the column for two minutes in order to thoroughly dry the resin. Discard the collection tube.

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 volume has not been eluted, spin the column at 14,000 x g (~12,000 RPM) for 1 additional minute.

D. Assessing RNA Yield and Quality by UV Absorbance

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

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

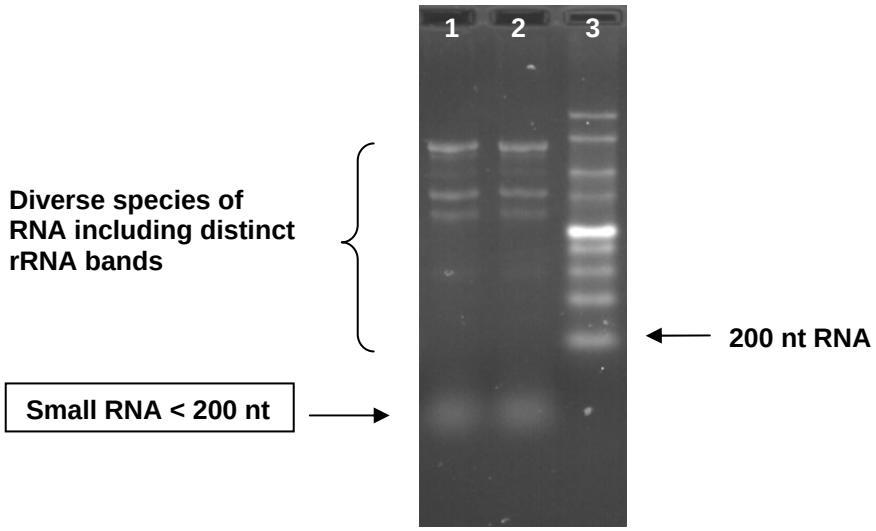
b. An A_{260} of 1.0 is equivalent to 40 μg RNA/mL. Calculate the RNA concentration in μg/mL as follows:

A_{260} x dilution factor x 40 = μg RNA/mL

c. The ratio of the readings at 260 and 280 nm (A_{260}/A_{280}) provides an estimate of the RNA purity with respect to contaminants that absorb in the UV range such as protein. Ratios of 1.8 to 2.1 indicate highly purified preparations of RNA. Contaminants such as protein that absorb at 280 nm will lower this ratio. However, RNA solutions with a ratio lower than 1.8 may still 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 > 1500 nucleotides in size or as sharp peaks in the electropherogram. It is common to see a diffuse smear of ethidium bromide staining between the distinct ribosomal bands, probably consisting of various mRNA species. Small RNA species such as tRNA 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 a 50 mg sample of Taxus (yew) needle using the SurePrep Plant/Fungi Total RNA Purification Kit. About one μg of total RNA isolated from yew needle was loaded in lanes 1 and 2 on a 2% MOPS-formaldehyde agarose gel. Lane 3 contained a 1kb RNA standard (BP2811). Electrophoresis was performed at 100 V for 90 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	Column has become clogged	Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. See also "Clogged Column" below.
	An alternative elution buffer was used	It is recommended that the **RNA 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 - 100% 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.
Clogged Column	Maximum number of cells or amount of tissue exceeds kit specifications	The optimal input is 50 mg of plant tissue or filamentous fungi, or 1 x 10^6 plant cells. However, for some species, up to 100 mg of tissue may be processed depending on the RNA content of the sample.
	Too much cell debris in the lysate supernatant	Ensure that most cell debris is removed in **Section II B**.
	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 does not perform well in downstream applications	RNA was not washed three times with the provided Wash Solution	Traces of salt from the binding step may remain in the sample if the column is not washed three times with the **Wash Solution**. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry spin under the Column Wash procedure is performed in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.

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
BP2814-25	SurePrep™ Water RNA/DNA Purification Kit
BP2815-50	SurePrep™ Soil DNA Isolation Kit
BP2816-50	SurePrep™ FFPE RNA Isolation Kit
BP2817-50	SurePrep™ Plant/Fungi Total RNA Purification Kit

B. Other Fisher BioReagents Functionally Tested for RNA Research

BP2484-50	Water, Sterile (DEPC-treated) 50mL
BP2484-100	Water, Sterile (DEPC-treated) 100mL
BP561-1	Water, Sterile (RNA Grade) 1L
BP2483-100	EDTA 0.5 M (DEPC-treated) 100mL
BP2483-1	EDTA 0.5 M (DEPC-treated) 1L
BP2483-500	EDTA 0.5 M (DEPC-treated) 500mL
BP2810-50	RiboLadder™ 100b RNA Standard with loading buffers
BP2811-50	RiboLadder™ 1Kb RNA Standard with loading buffers
BP3224-5	Optizyme™ Ribonuclease Inhibitor (Human Placental) 10,000U
BP3224-1	Optizyme™ Ribonuclease Inhibitor (Human Placental) 2,500U
BP3225-5	Optizyme™ Ribonuclease Inhibitor (Porcine) 10,000U
BP3225-1	Optizyme™ Ribonuclease Inhibitor (Porcine) 2,500U
BP3222-5	Optizyme™ Ribonuclease Inhibitor (Recombinant) 10,000U
BP3222-1	Optizyme™ Ribonuclease Inhibitor (Recombinant) 2,500U
BP3226-1	Optizyme™ Recombinant DNase I (RNase-free) 1,000U

BP3226-2	Optizyme™ Recombinant DNase I (RNase-free) 2,000U
BP176-100	2-Mercaptoethanol 100g
BP535-1	Lysozyme, Egg White 1g
BP535-5	Lysozyme, Egg White 5g
BP535-10	Lysozyme, Egg White 10g
BP2476-100	Tris-EDTA, 1X Solution, pH 7.4 100ml
BP2476-500	Tris-EDTA, 1X Solution, pH 7.4 500ml
BP160-100	Agarose, Low EEO, Multipurpose 100g
BP1360-100	Agarose, Low Melting, <1kb RNA 100g
BP1356-100	Agarose, Broad Separation Range for RNA 100g
BP1302-10	Ethidium Bromide, 1% Solution 10ml
BP2900-500	MOPS 10X Solution 500ml
BP2900-1	MOPS 10X Solution 1L

Fisher BioReagents Technical Support

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 Rev: 03/09