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Fisher BioReagents®

SurePrep™ Urine Exfoliated Cell RNA Purification Kit

Product Cat. # BP2803-50

Instruction Manual

I. Introduction

A. Product Description

The SurePrep™ Urine Exfoliated Cell RNA Purification Kit provides a rapid method for the isolation and purification of total RNA from exfoliated cells that have been shed into the urine from the urinary tract. RNA biomarkers from exfoliated cells can be used as non-invasive tools for a number of diagnostic and research applications including the diagnosis and monitoring of bladder, kidney, or other urinary-tract cancers. The kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). The RNA is preferentially purified from other cellular components such as proteins, as well as from contaminating chemical species found in urine such as glucose and salts, 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 other cellular components such as proteins without the use of phenol or chloroform. The process involves first lysing the exfoliated cells with the provided Lysis Solution (please see the flow chart on page 5). Ethanol is then added to the lysate, 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 twice with the provided wash solution in order to remove any remaining impurities, and the purified total RNA is eluted with the elution buffer.

C. Kit Specifications

Kit Specifications	
Column Binding Capacity	50 μg
Volume of Urine Processed	1 – 50 mL
Maximum Input of Exfoliated Cells	1 x 10^6 cells
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Time to Complete 10 Purifications	20 minutes
Average Yield	~ 1 μg RNA per 1 x 10^5 cells (varies due to cell density of sample)

D. Advantages

- Fast and easy processing using rapid spin-column format
- Isolate total RNA, from large mRNA down to microRNA (miRNA)
- RNA can be isolated and detected from as little as 100 exfoliated cells
- Isolate high quality total RNA from urine
- No phenol or chloroform extractions

E. Kit Components

Component	Catalog # BP2803-50 (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 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 Sheet (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™ Urine Exfoliated Cell RNA Purification Kit:

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

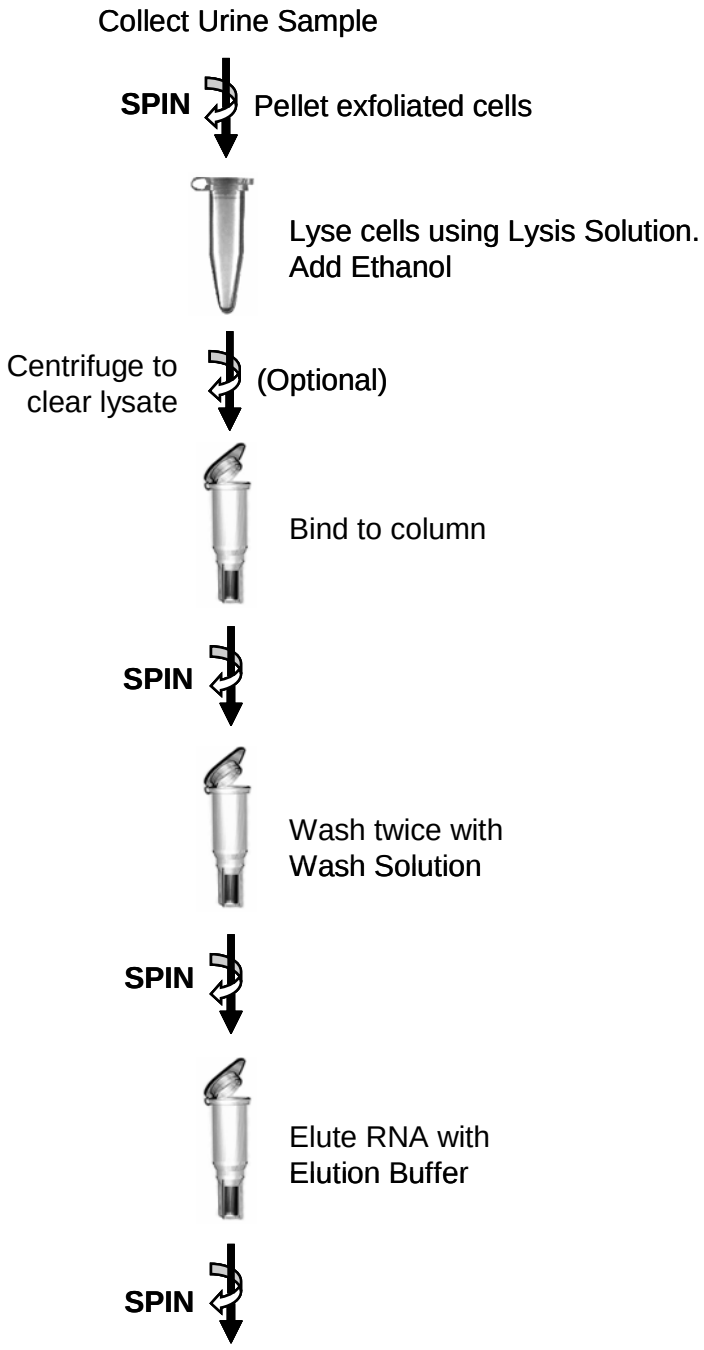
I. Working with RNA

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 Urine RNA using Fisher's Urine Exfoliated Cell RNA Purification Kit



Purified Total Urine 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 Exfoliated Cells in Urine

All centrifugation steps are carried out in a microcentrifuge at 14,000 x g (~ 12,000 RPM) except where noted. All centrifugation steps are performed at room temperature.

Notes Prior to Use

- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution** by adding 50 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution**. This will give a final volume of 72 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- 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. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
- Cell pellets can be flash-frozen using liquid nitrogen and stored at -70°C for later use or used directly in the procedure.
- Frozen pellets should not be thawed prior to beginning the protocol. Add the Lysis Solution directly to the frozen cell pellet.
- Frozen pellets should be stored for no longer than 2 weeks to ensure that the integrity of the RNA is not compromised.
- It is important to work quickly during this procedure.
- The maximum input of urine per column is 50 mL or 1 x 10^6 exfoliated cells.

Cell Lysate Preparation

a. Transfer 30 mL of urine to a 50 mL conical tube. Centrifuge the samples in a swinging bucket centrifuge at 650 x g for 5 minutes. The maximum input of urine is 50 mL or 1 x 10^6 cells per column. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.

Note: For samples less than 1.5 mL, transfer urine to a micro centrifuge tube and centrifuge at 650 x g (~2,000 RPM) for 5 minutes to pellet the cells. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.

b. 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. Transfer the lysate to a micro centrifuge tube.

c. Add 200 μL of 95 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.

d. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to step **III A** without centrifugation.

III. Purifying Total RNA from Sample Lysate

A. Binding RNA to Column

a. Assemble a spin column with one of the provided collection tubes.
b. Apply the clarified lysate with the ethanol onto the column and centrifuge for 1 minute.

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

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

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

B. Column Wash

a. Apply 500 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. Wash the column a second time by adding another 500 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.

C. 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 Buffer** to the column.
c. Centrifuge for 2 minutes at **200 x *g* (~1,500 RPM)**, followed by a 1 minute spin 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: A smaller volume of RNA Elution Buffer may be used in order to obtain a more concentrated sample. A minimum volume of 20 µL is recommended.

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 in TE (10 mM Tris and 1 mM EDTA, pH 7.4). Measure absorbance of the diluted sample in a 1 mL cuvette using a traditional UV-VIS spectrophotometer at 260 and 280 nm. The spectrophotometer should first be zeroed with the TE used to dilute the sample.

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

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

c. The ratio of the readings at 260 and 280 nm (A_{260}/A_{280}) provides an estimate of the RNA purity with respect to contaminants that absorb in the UV range such as protein. Ratios of 1.8 to 2.1 indicate highly purified preparations of RNA. Contaminants such as protein that absorb at 280 nm will lower this ratio. However, RNA solutions with a ratio lower than 1.8 may function well in downstream applications such as RT-PCR and Northern blotting.

E. Storage of RNA

The purified RNA sample may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

IV. Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Incomplete lysis of cells	Ensure that the appropriate amount of **Lysis Solution** was added to the cells.
	Column has become clogged	Do not exceed the recommended amounts of 50 mL of urine or 1 x 10^6 exfoliated cells. 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 **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% ethanol is added to the supplied **Wash Solution** prior to use.
	Low cell density in the sample	The cell number in different human urine samples can vary depending on the health of the individual. It is possible that the total RNA isolated is not visible when resolved on an agarose gel or detected by spectrophotometry. In such cases, a larger input volume may be used. Alternatively, a more sensitive method such as the Agilent BioAnalyzer or RT-PCR may be used for detection.
Clogged Column	Insufficient solubilization of cells	Ensure that the appropriate amount of **Lysis Solution** was added to the cells.
	Clarified lysate was not used for the binding step	Ensure that after the lysis step, the sample is centrifuged if visible precipitates form and only the clarified lysate is used in subsequent steps.
	High amounts of genomic DNA present in sample	The lysate may be passed through a 25 gauge needle attached to a syringe 5-10 times in order to shear the genomic DNA prior to loading onto the column.
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 20°C may cause precipitates to form that can cause the columns to clog.

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 "*Working with RNA"* at the beginning of this user guide.
	Procedure not performed quickly enough	In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly
	Improper storage of the purified RNA	For short term storage RNA samples may be stored at –20°C for a few days. It is recommended that samples be stored at –70°C for longer term storage.
	The cells are old	Older samples contain prematurely lysed cells which release RNase and can degrade RNA. Fresh urine samples are recommended.
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 **Wash Solution**. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
Genomic DNA contamination	Large amounts of starting material used	Perform RNAse-free DNaseI digestion on the RNA sample after elution to remove genomic DNA contamination (see Appendix A).

V. Related Products

A. Additional RNA Purification Kits

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

B. Other Fisher BioReagents Functionally Tested for RNA Research

BP2484-50	Water, Sterile (DEPC-treated) 50mL
BP2484-100	Water, Sterile (DEPC-treated) 100mL
BP561-1	Water, Sterile (RNA Grade) 1L
BP2483-100	EDTA 0.5 M (DEPC-treated) 100mL
BP2483-1	EDTA 0.5 M (DEPC-treated) 1L
BP2483-500	EDTA 0.5 M (DEPC-treated) 500mL
BP2810-50	RiboLadder™ 100b RNA Standard with loading buffers
BP2811-50	RiboLadder™ 1Kb RNA Standard with loading buffers
BP3224-5	Optizyme™ Ribonuclease Inhibitor (Human Placental) 10,000U
BP3224-1	Optizyme™ Ribonuclease Inhibitor (Human Placental) 2,500U
BP3225-5	Optizyme™ Ribonuclease Inhibitor (Porcine) 10,000U
BP3225-1	Optizyme™ Ribonuclease Inhibitor (Porcine) 2,500U
BP3222-5	Optizyme™ Ribonuclease Inhibitor (Recombinant) 10,000U
BP3222-1	Optizyme™ Ribonuclease Inhibitor (Recombinant) 2,500U
BP3226-1	Optizyme™ Recombinant DNase I (RNase-free) 1,000U
BP3226-2	Optizyme™ Recombinant DNase I (RNase-free) 2,000U
BP176-100	2-Mercaptoethanol 100g
BP535-1	Lysozyme, Egg White 1g
BP535-5	Lysozyme, Egg White 5g
BP535-10	Lysozyme, Egg White 10g
BP2476-100	Tris-EDTA, 1X Solution, pH 7.4 100ml
BP2476-500	Tris-EDTA, 1X Solution, pH 7.4 500ml
BP160-100	Agarose, Low EEO, Multipurpose 100g
BP1360-100	Agarose, Low Melting, <1kb RNA 100g
BP1356-100	Agarose, Broad Separation Range for RNA 100g
BP308-100	MOPS 100g
BP308-500	MOPS 500g

VI. Appendix A

Protocol for Optional On-Column DNA Removal

The SurePrep™ Urine Exfoliated Cell RNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional protocol is provided below for maximum removal of residual DNA that may affect sensitive downstream applications. It is recommended that an RNase-free DNase I is 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 procedure up to and including "**Binding RNA to Column**" (step **III A**).

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 above to the column. Centrifuge for 30 seconds at 200 x g (~1500 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^{o}C for 15 minutes.

6. Without further centrifugation, proceed directly to "**Column Wash**" (step **III B**).

Technical Support

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