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Fisher BioReagents®

SurePrep™ Leukocyte RNA Purification Kit

Product Cat. # BP2807-50

Instruction Manual

I. Introduction

A. Product Description

The SurePrep™ Leukocyte RNA Purification Kit provides a rapid method for the isolation and purification of total leukocyte (white blood cell) RNA from mammalian blood samples. RNA isolated from blood can be used in various expression studies including those focusing on diseases. However, a major problem with blood RNA isolation is that a large portion of the RNA present is globin mRNA, which is found primarily in red blood cells. In fact, up to 70% of the mass of mRNA in whole blood total RNA is globin transcripts. Therefore, it is desirable to be able to remove the red blood cells from the sample and isolate only the RNA associated with the leukocytes which will result in improved expression profiling and other applications by removing the masking effects of this abundant globin mRNA. Fisher's Leukocyte RNA Purification Kit can be used to isolate and purify total leukocyte RNA, including all small RNAs, from mammalian blood samples.

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 the other cellular components such as proteins without the use of phenol or chloroform. For leukocyte RNA purification, whole blood samples are first collected with anticoagulants. The red blood cells are removed through differential red blood cell lysis, and the leukocytes are recovered by centrifugation (please see flow charts on pages 5 and 6). The recovered leukocytes are then lysed, and the lysate is loaded onto a supplied 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 three times with the provided wash solution in order to remove any remaining impurities, and the purified leukocyte RNA is eluted with the elution buffer. The SurePrep™ kit allows for the isolation of total leukocyte RNA including all small RNA species. 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 Blood Input	2 mL or 3 x 10^6 Leukocytes
Minimum Blood Input	10 μL
Time to Complete Ten Purifications	45 minutes
Average Yield: 500 μL human blood	1.5 μg

D. Advantages

- Fast and easy processing using rapid spin-column format
- No phenol or chloroform extractions
- Differential red blood cell lysis allows for the removal of a majority of globin mRNAs
- Isolate total leukocyte RNA, including all small RNA species
- High quality leukocyte RNA can be used in a number of downstream applications

E. Kit Components

Component	Catalog # BP2807-50 (50 preps)
RBC Lysis Solution	180 mL
Binding Solution	25 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. The RBC Lysis Solution should be stored at 4°C upon arrival. These reagents should remain stable for at least 2 years in their unopened containers.

G. Precautions and Disclaimers

User must determine the suitability of the product for their particular use. This kit is intended for research purposes only and not for human or drug use. This kit is not designed for diagnostic purposes. 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.

Ensure that a proper lab coat, disposable gloves and protective eyewear are worn when working with this kit.

H. Customer-Supplied Reagents and Equipment

You must have the following in order to use the Leukocyte 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 1
Procedure for Differential Red Blood Cell (RBC) Lysis

Collect Blood in 4.8mM EDTA

Add 5 Volumes of **RBC Lysis Buffer.**
Vortex and incubate for 3-5 minutes.

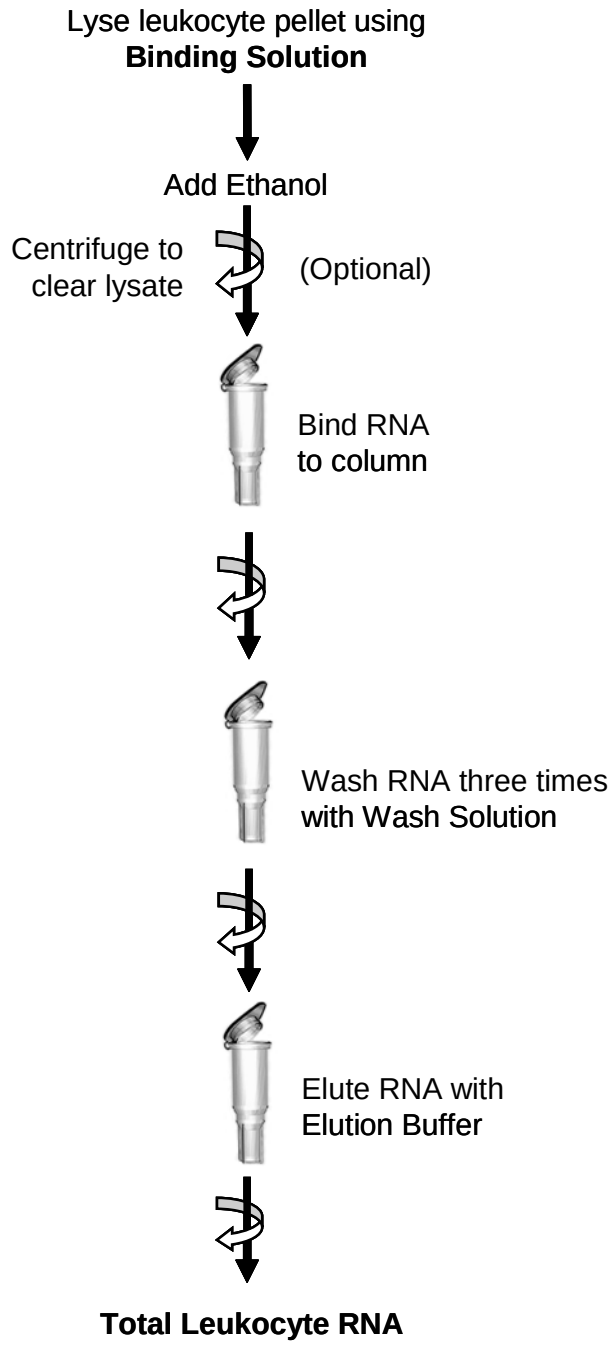
1. Centrifuge to pellet cells
2. Gently decant supernatant

Add 2 Volumes of **RBC Lysis Buffer.**
Vortex

1. Centrifuge to pellet cells
2. Gently decant supernatant

White Leukocyte Pellet

Flow Chart 2
Procedure for Leukocyte Total RNA Purification



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 Leukocytes

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 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 **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.
- It is recommended that no more than 2 mL of blood be used in order to prevent possible clogging of the column.
- 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.
- Blood samples should be collected into a tube containing EDTA such that the final concentration of the EDTA is ~ 4.8 mM.
- Only fresh blood can be used with this procedure. Frozen whole blood can not be used.
- For optimal results, blood samples should be processed within a few hours of collection.
- Leukocyte pellets generated in the first step can be used directly in the procedure, or stored at -70°C for later use. Pellets should be stored for no longer than 2 weeks to ensure that the integrity of the RNA is not compromised.
- Frozen leukocyte pellets should not be thawed prior to beginning the protocol. Add the **Binding Solution** directly to the frozen pellet.
- It is important to work quickly during this procedure.

Red Blood Cell Lysis

a. Add 5 volumes of **RBC Lysis Solution** to blood samples collected with EDTA. (i.e., add 2.5 mL of **RBC Lysis Solution** to 500 µL of blood).
b. Incubate at room temperature for 3 to 5 minutes, with brief vortexing during the incubation to mix.

   Note: Ensure that the solution changes from a milky, opaque pink to clear red before proceeding to the next step.

c. Centrifuge at 250 x g (~2,000 RPM) for 3 minutes and decant supernatant.
d. Add 2 additional volumes of **RBC Lysis Solution** to pelleted white blood cells and mix by gentle vortexing for 10 seconds. (i.e. add 1 mL of **RBC Lysis Solution** to every 500 µL of input blood volume).
e. Centrifuge at 250 x g (~2,000 RPM) for 3 minutes and decant supernatant. A few µL of media may be left behind with the pellet in order to ensure that the pellet is not dislodged.

   Note: The leukocyte pellet should be white. If the pellet is red, then the red blood cell lysis procedure was incomplete. Please refer to the troubleshooting guide at the back of the manual if this occurs.

Cell Lysate Preparation

a. Add 350 µL of **Binding Solution** directly to pelleted leukocytes.
b. Lyse cells by gentle vortexing until homogeneity is reached.
c. Add 200 µL of 95 – 100% ethanol (provided by the user) to the mixture and mix by vortexing for 10 seconds.

   Note: For input amounts greater than 500 µL of blood or 10^6 leukocytes, 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.

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 column with one of the provided collection tubes.
b. Apply the clarified lysate onto the column and centrifuge for 1 minute.
c. Discard the flowthrough. Reassemble the spin column with its collection tube.

Optional Step: The SurePrep™ Leukocyte RNA Purification Kit isolates total leukocyte 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.

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** and **b** to wash column a second time.
d. Wash column a third time by adding another 400 µL of **Wash Solution** and centrifuging for 2 minutes.
e. Ensure that the column is dry. Spin for an additional minute, if necessary.
f. Discard the collection tube with the 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 one additional minute.

   Note: For maximum RNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (repeat steps **b** and **c**).

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:

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

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

E. 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 leukocytes	Ensure that the appropriate amount of **Binding Solution** was used to lyse the leukocyte pellet.
	Lysis of red blood cells was incomplete	Ensure that the blood sample is collected with the appropriate amount of EDTA, which will prevent coagulation of the red blood cells and allow for proper lysis. Also check that the appropriate amount of **RBC Lysis Solution** is added to the blood sample, and that it is mixed and incubated properly.
	Ethanol was not added to the lysate	Ensure that 200 µL of 95-100% 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.
	An alternative elution solution was used	It is recommended that the **RNA Elution Buffer** supplied with this kit be used for maximum RNA recovery.
	The column has become clogged	Do not exceed 2 mL of blood or 3 x 10^6 leukocytes per column. The amount of blood used may need to be decreased if the column shows clogging below the recommended level. See also "Clogged Column" below.
Clogged Column	Incomplete lysis of leukocytes	Ensure that the appropriate amount of **Binding Solution** was used to lyse the leukocyte pellet.
	Lysis of red blood cells was incomplete	Ensure that the blood sample is collected with the appropriate amount of EDTA which will prevent coagulation of the red blood cells and allow for proper lysis. Improperly lysed red blood cells will clog the column.
	Amount of blood used exceeds kit specifications	It is recommended that no more than 2 mL of blood or 3 x 10^6 leukocytes be used in order to prevent possible clogging of the column.
	Clarified lysate was not used for the binding step	Ensure that the sample is centrifuged for at least 1 minute after lysis, and that only the clarified lysate is applied to 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
Cloudy Pink Solution Does Not Become Clear Red During RBC Lysis	Incomplete red blood cell lysis	The solution should become a translucent red colour after **RBC Lysis Solution** has been added and incubated with the blood. If not, pellet the leukocytes and remove as much of the supernatant as possible. Add another 5 volumes of RBC Lysis solution and incubate again.
Leukocyte pellet is red	Incomplete red blood cell lysis	The leukocyte pellet should be white, with only residual traces of red blood cells. If red blood cell lysis is incomplete, the pellet will be red. In this case resuspend the leukocyte pellet in another 5 volumes of **RBC Lysis Solution** and incubate at room temperature for another 5 minutes.
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.
	Leukocyte pellets were too old	Leukocyte pellets generated at the end of step **II B** may be stored for up to 2 weeks at -70°C and used in this procedure. It is not recommended that samples be frozen for longer than 2 weeks, as the integrity of the RNA will be compromised.
RNA does not perform well in downstream applications	RNA was not washed 3 times with the provided Wash Solution	Traces of salt from the binding step may remain in the sample if the column is not washed 3 times with **Wash Solution**. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
Residual genomic DNA contamination	Large amounts of genomic DNA in starting material	Perform RNase-free DNaseI digestion on the RNA sample after elution to remove genomic DNA contamination.

V. Related Products

A. Additional RNA Purification Kits

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

B. Other Fisher BioReagents Functionally Tested for RNA Research

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

VI. Appendix A

Protocol for Optional On-Column DNA Removal

The SurePrep™ Leukocyte RNA Purification Kit isolates leukocyte 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 Leukocyte RNA Isolation 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 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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