NORGEN BIOTEK CORPORATION

3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

Leukocyte RNA Purification Kit — Product Insert

Product # 21200

Norgen's 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. Norgen's Leukocyte RNA Purification Kit can be used to isolate and purify total leukocyte RNA, including all small RNAs, from mammalian blood samples.

Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's 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 4 and 5). The recovered leukcoytes are then lysed, and the lysate is loaded onto a supplied spin column. Norgen'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 A in order to remove any remaining impurities, and the purified leukocyte RNA is eluted with the Elution Solution A. Norgen's 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.

Specifications

Kit Specifications	
Maximum Column Binding Capacity	50 μg
Maximum Column Loading Volume	650 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 10 Purifications	40 minutes
Average Yield: 500 μL human blood	1.5 μg

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. The RBC Lysis Buffer should be stored at 4°C upon arrival. These reagents should remain stable for at least 1 year in their unopened containers.

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

Kit Components

Component	Product # 21200 (50 preps)
RBC Lysis Buffer	2 x 100 mL
Buffer RL	30 mL
Wash Solution A	38 mL
Elution Solution A	6 mL
Mini Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

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. MSDS sheets are available upon request.

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.

Customer-Supplied Reagents and Equipment

You must have the following in order to use the Leukocyte RNA Purification Kit:

- Benchtop microcentrifuge
- β-mercaptoethanol
- 96 - 100% ethanol

Working with RNA

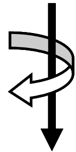
RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense 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

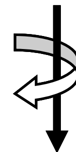
Collect Blood in 4.8mM EDTA

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 Total Leukocyte RNA Purification

Lyse leukocyte pellet using **Buffer RL**

Add Ethanol

Bind RNA to column

Wash RNA three times with Wash Solution A

Elute RNA with Elution Solution A

Total Leukocyte RNA

Procedure

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:

$$\text{RPM} = \sqrt{\frac{\text{RCF}}{(1.118 \times 10^{-5})\,(r)}}$$

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.

Protocol for Total RNA Purification from Isolated Leukocytes

Notes Prior to Use

- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~14,000 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 A** by adding 90 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution A**. This will give a final volume of 128 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 **Buffer RL** by adding 10 μL of β-mercaptoethanol (provided by the user) to each 1 mL of **Buffer RL** 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 Buffer directly to the frozen pellet **(Step 2a)**.
- It is important to work quickly during this procedure.

1. **Red Blood Cell Lysis**
   a. Add 5 volumes of **RBC Lysis Buffer** to blood samples collected with EDTA. (i.e.: Add 2.5 mL of **RBC Lysis Buffer** 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 Buffer** to pelleted white blood cells and mix by gentle vortexing for 10 seconds. (i.e. Add 1 mL of **RBC Lysis Buffer** 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.

2. **Cell Lysate Preparation**
   a. Add 350 μL of **Buffer RL** directly to pelleted leukocytes.
   b. Lyse cells by gentle vortexing until homogeneity is reached.
   c. Add 200 μL of 96 – 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.

3. **Binding to Column**
   a. Assemble a column with one of the provided collection tubes.
   b. Apply the lysate with the ethanol onto the column and centrifuge for 1 minute at **3,500 x *g* (~6,000 RPM)**.

   Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute at **14,000 x *g* (~14,000 RPM)**.

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

Optional Step:

Norgen's 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. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step.

4. **Column Wash**
   a. Apply 400 μL of **Wash Solution A** to the column and centrifuge for 1 minute.

      Note: Ensure the entire Wash Solution A 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 **4a** and **4b** to wash column a second time.
d. Wash column a third time by adding another 400 μL of **Wash Solution A** and centrifuging for 1 minute.
e. Discard the flowthrough and reassemble the spin column with its collection tube.
f. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

5. **RNA Elution**
   a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
   b. Add 50 μL of **Elution Solution A** to the column.
   c. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by a 1 minute spin at **14,000 x *g* (~14,000 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 (~14,000 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 5b and 5c**).

6. **Storage of RNA**
   a. 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.

Appendix A

Protocol for Optional On-Column DNA Removal

Norgen's 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 Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step.

1. For every on-column reaction to be performed, prepare a mix of 15 μL of **DNase I** and 100 µL of **Enzyme Incubation Buffer** using Norgen's RNase-Free DNase I Kit (Product # 25710). Mix gently by inverting the tube a few times. **DO NOT VORTEX**.

   Note: If using an alternative DNase I, 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.

2. Perform the Leukocyte RNA Isolation Procedure up to and including "**Binding to Column**" (Step 3).
3. Apply 400 μL of **Wash Solution A** 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 and centrifuge at 14, 000 x g (~14 000 RPM) for 1 minute.

   Note: Ensure that the entire DNase I solution passes through the column. If needed, spin at 14, 000 x g (~14 000 RPM) for an additional minute.

5. After the centrifugation in Step 4, pipette the flowthrough that is present in the collection tube back onto the top of the column.

   Note: Ensure Step 5 is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA, in particular for small RNA species.

6. Incubate the column assembly at 25 - 30°C for 15 minutes.
7. Without further centrifugation, proceed directly to the second wash in the "**Column Wash**" section (Step 4c).

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Incomplete lysis of leukocytes	Ensure that the appropriate amount of Buffer RL 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 Buffer 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 96-100% ethanol is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution A	Ensure that 90 mL of 96 - 100% ethanol is added to the supplied Wash Solution A prior to use.
	An alternative elution solution was used	It is recommended that the Elution Solution A 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 Buffer RL 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.
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°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 1 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 may be compromised.
RNA does not perform well in downstream applications	RNA was not washed 3 times with the provided Wash Solution A	Traces of salt from the binding step may remain in the sample if the column is not washed 3 times with Wash Solution A. 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. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step.

Related Products	Product #
RNase-Free DNase I Kit	25710
Total RNA Purification Kit	17200
Cytoplasmic & Nuclear RNA Purification Kit	21000
microRNA Purification Kit	21300
100b RNA Ladder	15002
1kb RNA Ladder	15003

Technical Support
Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6
Phone: (905) 227-8848
Fax: (905) 227-1061
Toll Free in North America: 1-866-667-4362

© 2014 Norgen Biotek Corp.