

Leukocyte RNA Purification Kit

Product # 21200

Product Insert

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 following pages). The recovered leukocytes 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 and genomic DNA 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 buffer in order to remove any remaining impurities, and the purified leukocyte RNA is eluted with the elution buffer. 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, RNA protection and primer extension, and expression array assays.

Specifications

Kit Specifications	
Column Binding Capacity	50 µg
Maximum Column Loading Volume	700 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Maximum Blood Input	500 µL
Minimum Blood Input	10 µL
Time to Complete 10 purifications	45 minutes
Average Yield: 500 µL human blood	1.5 µg

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 samples)
RBC Lysis Buffer	180 mL
Binding Solution	25 mL
Wash Solution	50 mL
Elution Buffer	6 mL
Micro Spin Columns (assembled with 2 mL collection tubes)	50
Elution tubes (1.7 mL)	60
Product Insert	1

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 1 year in their unopened containers.

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
- 95 - 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 **Binding Solution**



Add Ethanol

Centrifuge to
clear lysate



Bind RNA

SPIN



Wash RNA

SPIN



Elute RNA

SPIN



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:

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

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.

Protocol for Total RNA Purification from Isolated Leukocytes

All centrifugation steps are carried out in a benchtop microcentrifuge at $14,000 \times g$ ($\sim 14,000$ 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 35 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution**. This will give a final volume of 50 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 Buffer** by adding 10 μ L of β -mercaptoethanol (provided by the user) to each 1 mL of **Binding Buffer** required. β -mercaptoethanol is toxic and should be dispensed in a fume hood.
- It is recommended that no more than 500 μ L of blood be used in order to prevent possible clogging of the column.
- 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 $-70^{\circ}C$ for later use. Pellets should be stored for no longer than 2 weeks to ensure that the integrity of the RNA is not compromised.
- 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.
- 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.
- 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 if this occurs.

2. 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.
- d. Spin lysate for 2 minutes in a benchtop microcentrifuge to pellet any cell debris.

3. Binding to Column

- a. Apply the clarified lysate (from step **2d**) onto the column and centrifuge for 1 minute.
- b. Discard the flowthrough. Reassemble the spin column with its collection tube.

4. 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. Wash column a second time by adding another 400 μL of **Wash Solution** and centrifuging for 2 minutes.
- c. Ensure that the column is dry. Spin for an additional minute, if necessary.
- d. Discard the collection tube with the flowthrough.

5. RNA Elution

- a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
- b. Add 50 μ L of **Elution Buffer** to the column.
- c. Centrifuge for 3 minutes at **5,200 x g (~8,000 RPM)**. Note the volume eluted from the column. If the entire 50 μ L 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.

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 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 μ L of 95-100% ethanol is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution	Ensure that 35 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 Elution Buffer supplied with this kit be used for maximum RNA recovery.
	The column has become clogged	Do not exceed 500 μ L of blood 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 500 μ L of blood 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 2 minutes 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 “ <i>Working with RNA</i> ” 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 will be compromised.
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.
Residual genomic DNA contamination	Large amounts of genomic DNA in starting material	Perform DNaseI digestion on the RNA sample after elution to remove genomic DNA contamination.

Related Products	Product #
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.

344 Merritt St., St. Catharines, ON Canada L2T 1K6
Phone: (905) 227-8848
Fax: (905) 227-1061
Toll Free in North America: 1-866-667-4362