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Leukocyte RNA Purification Plus Kit — Product Insert

Product # 21250

Norgen's Leukocyte RNA Purification Plus Kit provides a rapid method for the isolation and purification of total leukocyte (white blood cell) RNA from up to 3 mL of 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 Plus 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 3 and 4). The recovered leukcoytes are then lysed, and the lysate is loaded onto a Lysate Homogenization Column. Next, ethanol is added to the lysate and the lysate is applied onto a provided Single Cell RNA 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 once with the provided Wash Solution A, followed by DNase I treatment in order to remove any traces of DNA from the RNA preparation. Next, the RNA is washed twice with the provided Wash Solution two times in order to remove any remaining impurities, followed by a dry spin. The purified leukocyte RNA is then 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	10 µg
Maximum Column Loading Volume	650 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Maximum Blood Input	3 mL
Time to Complete 10 Purifications	40 minutes

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 1 year after the date of shipment. The RBC Lysis Buffer should be stored at 4°C upon arrival.

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 # 21250 (50 preps)
RBC Lysis Buffer	2 x 1 L
Buffer RL	30 mL
Wash Solution A	38 mL
Elution Solution A	6 mL
Enzyme Incubation Buffer	6 mL
DNase I	1 Vial
Lysate Homogenization Column	50
Single Cell RNA Column	50
Collection Tubes	100
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.

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. The **Buffer RL** contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

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 Plus Kit:

- Centrifuge with a swinging bucket rotor
- Benchtop microcentrifuge
- β-mercaptoethanol
- 96 - 100% ethanol
- 70% 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 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 and gentle vortexing

Pass lysate through Lysate Homogenization Column

Add ethanol.
Bind RNA to Single Cell RNA Column

Wash with Wash Solution A

Treat with DNase I

Wash with Wash Solution A twice.
Dry spin

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:

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

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

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.
- (Optional) 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.
- 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 and sodium citrate
- 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 after the addition of Buffer RL for later use.

1. **Red Blood Cell Lysis**
   a. Add 5 volumes of **RBC Lysis Buffer** to blood samples collected with EDTA. (i.e.: Add 15 mL of **RBC Lysis Buffer** to 3 mL 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 dark transparent red before proceeding to the next step.

   c. Centrifuge at 200 x g (~1,000 RPM) for 3 minutes in a swing bucket centrifuge 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 6 mL of **RBC Lysis Buffer** to every 3 mL of input blood volume)

e. Centrifuge at 200 x g (~1,000 RPM) for 3 minutes in a swing bucket centrifuge 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, repeat Step 1d to 1e once.

2. Cell Lysate Preparation

a. Add 600 μL of **Buffer RL** directly to pelleted leukocytes.
b. Lyse cells by gentle vortexing until homogeneity is reached.

3. Lysate Homogenization

a. Assemble a Lysate Homogenization Column (with Blue Ring) with a Collection Tube.
b. Apply the lysate onto the column and centrifuge for 3 minutes at **14,000 x *g* (~14,000 RPM)**.
c. Retain the flowthrough and measure the volume.

4. Binding to Column

a. To the lysate from Step 3c, add an equal volume of 70% ethanol (~ 600 μL).
b. Assemble a Single Cell RNA Column with one of the provided collection tubes.
c. Apply up to 650 μL of 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)**.

d. Discard the flowthrough. Reassemble the spin column with its collection tube.
e. Repeat Steps 4c and 4d until all lysate with ethanol has passed through the column.

5. First Column Wash and DNase I Treatment

a. Apply 650 μL of **Wash Solution A** to the column and centrifuge at 14, 000 x g (~14 000 RPM) 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. For every on-column reaction to be performed, prepare a mix of 10 μL of **DNase I** and 60 μL of **Enzyme Incubation Buffer**. **DO NOT VORTEX**.
d. Apply 70 μL of the RNase-free DNase I solution prepared in Step 5c to the column and centrifuge at 14, 000 x g (~14 000 RPM) for 1 minute.
e. Incubate the column assembly at 25 - 30°C for 15 minutes.

6. Post-DNase I Column Washes and Dry Spin

a. Without further centrifugation, apply 650 μL of **Wash Solution A** to the column and centrifuge at 14, 000 x g (~14 000 RPM) for 1 minute.
b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Apply 650 μL of **Wash Solution A** to the column and centrifuge at 14, 000 x g (~14 000 RPM) for 1 minute.
d. Discard the flowthrough and reassemble the spin column with its collection tube.

e. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

7. RNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.

b. Add 10 – 20 µL of **Elution Solution A** to the column.

c. Centrifuge for 1 minute at **200 x *g* (~2,000 RPM)**, followed by a 2 minute spin at **14,000 x *g* (~14,000 RPM).** Note the volume eluted from the column.

Note: For maximum RNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 5b and 5c**).

8. 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 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 an equal volume 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 3 mL 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.

Problem	Possible Cause	Solution and Explanation
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 3 mL of blood 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.
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.

Problem	Possible Cause	Solution and Explanation
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.

Norgen's purification technology is patented and/or patent pending. See www.norgenbiotek.com/patents

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