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RBC Lysis Buffer

Product Insert

Product # 21201 (90 mL)

Norgen's RBC Lysis Buffer is designed for the differential lysis of red blood cells present in whole blood samples. The removal of red blood cells from whole blood is often desirable, particularly during the study of leukocyte DNA, proteins or RNA. Abundant proteins (including albumin) and RNAs (including globin mRNA) that are present in red blood cells and may interfere with downstream applications such as expression analysis are effectively removed during this process. For the procedure, whole blood samples are first collected in the presence of anticoagulants. The red blood cells are then removed through lysis using the RBC Lysis Buffer, and the leukocytes which remain can then be recovered by centrifugation. Genomic DNA, proteins or RNA can then be isolated from the purified leukocytes, as Norgen's RBC Lysis Buffer is RNAase and DNase free.

Product Components

Component	Product # 21201
Cell Lysis Reagent	90 mL
Product Insert	1

Storage Conditions and Product Stability

The RBC Lysis Buffer should be stored at 4°C. This reagent should remain stable for at least 1 year in its unopened container.

Precautions and Disclaimers

This reagent 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 Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- Anticoagulant (ex: EDTA)

Flow Chart

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

Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge at 250 x g (~2,000 RPM), so please check your microcentrifuge specifications to ensure that it is capable of the proper speed. 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.

Notes Prior to Use

- All centrifugation steps are carried out in a benchtop microcentrifuge at 250 x g (~2,000 RPM). All centrifugation steps are performed at room temperature.
- Ensure that the solution is at room temperature prior to use.
- 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 can be used directly, 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.

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.

Troubleshooting Guide

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.

Related Products	Product #
Leukocyte RNA Purification Kit	21200
Blood Genomic DNA Isolation Kit	18200

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.

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