PROMETHEUS™
Protein Biology Products

Instruction Manual

RBC Lysis Buffer

Catalog No.: 18-448, 18-449 & 18-450

Introduction

We have developed the Red Blood Cell (RBC) Lysis Buffer, which provides a quick and optimal lysis of RBCs with minimal effects on leukocytes of human blood samples. This buffer is not intended for use with whole blood from any other species. Human whole blood is composed of about 45% red blood cells and it is difficult to analyze the phenotype and function of leukocytes in whole blood without removing the red blood cells. Our RBC Lysis Buffer is ammonium chloride-based, ready to use solution, which can be used for DNA/RNA isolation and flow cytometry experiments using human blood samples. The supplied RBC Lysis Buffer does not contain any fixative so the cells remain viable after red blood cell lysis. Our RBC Lysis Buffer eliminates the need for hazardous organic extractions or chaotropic agents and is particularly useful for high-volume research, which currently requires Ficoll/ Hypaque gradients.

Buffer Components

Name	Cat. No. 18-448	Cat. No. 18-449	Cat. No. 18-450	Storage Temp*
RBC Lysis Buffer	125 ml	250 ml	500 ml	4-8°C

**The RBC Lysis Buffer is shipped at ambient temperature. Upon receiving, store it refrigerated at* 4-8°C.

IMPORTANT NOTE ABOUT THE PRODUCT:

- *Our RBC Lysis Buffer is designed for in vitro Research Use Only and must not be used for diagnostics/ therapeutic purposes in humans or animals.*
- *Follow all safety precautions for handling the biohazardous materials.*
- *Properly dispose of all contaminated materials following the state and federal laws.*
- *Decontaminate the work surfaces and use a Biosafety cabinet whenever aerosols might be generated.*

Items Needed, But Not Supplied with the Product

The supplied product contains only RBC Lysis Buffer and following items may be needed, depending on the experiment:

- Molecular Grade or Double Distilled Water
- DNA/RNA Extraction Buffers/ Reagents/Columns
- Flow Cytometry Staining Buffers/ Reagents and antibodies

Preparation Before Use

I. Use blood, stored in an anticoagulant, such as EDTA, or Heparin.
II. The blood sample shouldn't be more than a month old and for best results in DNA applications, use fresh blood or blood stored for less than 3 days and for RNA work use the fresh blood sample.
III. Warm the blood sample to room temperature (RT; +15 to +25^{0}C).
IV. Warm the RBC Lysis Buffer to RT.

Protocol(s)

For use in DNA/RNA Extraction

Our RBC Lysis Buffer is useful for both DNA and RNA isolation, as it's designed for the preferential lysis of red blood cells (RBCs) from human whole blood, yielding intact white blood cells that are free of RBCs for further applications.

RBC Lysis from Whole Blood

1. Take 500 µl of whole blood in a 1.5 ml microfuge tube and add 1 ml of RBC Lysis Buffer. Cap the tube and mix it by gently inverting the tube. DO NOT VORTEX.
2. Incubate the tube at RT (+15 to +25^{0}C) for 10 minutes and invert the tubes a few times while incubating.
3. Centrifuge the tube at 500 x g for 5 minutes in a Microcentrifuge at RT.
4. Using a sterile pipette tip, carefully remove the clear red supernatant and dispose of properly as a BIOHAZARD

5. After the removal of the supernatant, a white pellet should be visible in the bottom of the tube. If traces of RBCs are observed, repeat the above lysis and centrifugation steps to remove.
6. Resuspend the white pellet in an appropriate buffer or 1X PBS for further experiment/ analysis.

RBC Lysis from tissues/ solid tumor samples

1. Dissociate the tissue or solid tumor samples into single cells and centrifuge at 500 x g for 5 minutes and carefully remove the supernatant.
2. Resuspend the cell pellet in RBC Lysis buffer and incubate for 10 minutes at RT and invert the tube a few times while in incubation.
3. Centrifuge at 500 x g for 5 minutes at RT and remove the supernatant. Carefully dispose of the supernatant as a BIOHAZARD.
4. Resuspend the cell pellet in appropriate buffer and the sample is ready for further analysis.

For use in Flow-cytometry

Our RBC Lysis Buffer lyse the red blood cells, which results in good red blood cell debris and light scatter separation of lymphocytes, when analyzed by flow-cytometry. The protocol has been optimized for blood samples and if the intended use is for any other sample, it is recommended that the researcher optimize the conditions for best results and use their own established protocol.

1. Aliquot 100 µl sample of whole blood to a tube and add 2 ml of RBC Lysis Buffer (RT) and invert the tubes a few times or pulse vortex for < 5 seconds.
2. Incubate the tubes for 15 minutes at RT.
3. Centrifuge the tube at 500 x g for 5 minutes at RT and carefully remove the supernatant using a sterile pipette tip, without disturbing the pellet.
4. If traces of RBCs are observed, repeat the lysis and centrifugation steps to remove.
5. Re-suspend the cells in appropriate volume of buffer for further analysis.

RELATED PRODUCTS:

1. **Protein Extraction Buffers/ Kits (Cat. No. 18-400, 18-402, 18-404, 18-406, 18-409, 18-411**
   For extracting proteins from Bacteria, Insects Cells, Mammalian cells, Tissues and Yeast samples.
2. **RIPA Lysis Buffer (18-415, 18-416 and 18-417:**
   For extracting proteins from different species samples.
3. **Protein Loading Buffer [2X], Cat. No. 20-309:**
   Non-reducing ready to use buffer for loading protein samples on to the gel. Premixed, just add an equal volume to your protein sample.
4. **Protein Loading Buffer [2X], Cat. No. 20-310:**
   Reducing ready to use buffer for loading protein samples on to the gel. Premixed, just add an equal volume to your protein sample.

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