1X RBC lysis buffer

Cat# C1064-1L

Store at 2-8°C

INTRODUCTION

This 1X Red Blood Cell (RBC) Lysis Buffer is formulated for optimal lysis of erythrocytes in single-cell suspensions of mouse hematopoietic tissues such as spleen and human peripheral blood. This buffer contains ammonium chloride, which lyses red cells with minimal effect on lymphocytes when used as instructed. Nucleated red cells are not effectively lysed with ammonium chloride.

Product components

C1064 1X RBC lysis buffer 1L	
NH_4Cl	150 mM
$KHCO_3$	10 mM
$EDTA.Na_2 \cdot 2H$	1 mM

Applications Tested

The 1X RBC Lysis Buffer has been tested on normal human peripheral blood and mouse splenocytes followed by flow cytometric analysis.

For sterile use, please filter through 0.22 µm membrane.

Protocol A: Using 1X or 10X RBC Lysis buffers

Both the 1X and 10X RBC Buffers are designed to lyse RBC in whole blood (using heparin or EDTA as the anti-coagulant) or tissue preparations using ammonium chloride-based osmotic shock. The 10X RBC Lysis Buffer (ACE Biolabs #C1066) is specially formulated for optimal lysis of RBC in peripheral blood. It has been validated to work on whole blood from human, mouse, rat, canine and non-human primate sources. The 1X RBC Lysis Buffer is optimized for lysis of RBC in human peripheral blood or single-cell suspensions of mouse hematopoietic tissues such as spleen or bone marrow.

General notes:

1. Before use, the 10X RBC Lysis Buffer (ACE Biolabs #C1066) must be diluted 1:10 with room temperature, reagent-grade water.
2. The 10X RBC Lysis Buffer (ACE Biolabs #C1066) has been shown to work equivalently in blood collected using either heparin or EDTA as the anti-coagulent.
3. In general, a small number of residual RBC does not interfere with subsequent use of cells and can be gated out during flow cytometric analysis; however, a second round of lysis can be performed, if desired.

Materials:

1. 1X PBS
2. 10X RBC Lysis Buffer (ACE Biolabs #C1066) or 1X RBC Lysis Buffer
3. 50-mL conical tubes
4. Flow Cytometry Staining Bufferor other buffer of choice
5. 12 x 75 mm round-bottom test tubes
6. Primary antibodies (directly conjugated)

Lysis of mouse splenocytes

1. Harvest mouse spleen and prepare a single cell suspension.
2. Pellet the cells by centrifugation (300-400 x g) at 2-8°C and aspirate the supernatant.
3. Resuspend the pellet with 5 mL of 1X RBC Lysis Buffer per spleen.
4. Incubate at room temperature for 4-5 minutes with occasional shaking (this step may also be performed on ice).
5. Stop the reaction by adding 20-30 mL of 1X PBS.
6. Spin the cells (300-400 x g) at 2-8°C and resuspend the pellet in an appropriate buffer for use in the next step of your experimental procedure. 7. Perform a cell count at this time.

Note: In general a small number of residual red cells does not interfere with the proliferation assays and can be gated out from flow cytometric analysis. However, if required, a second round of lysis can be performed.

Lysis of mouse blood

1. Add 10 mL of 1X RBC Lysis Buffer per 1 mL of mouse blood.
2. Incubate at room temperature for 4-5 minutes with occasional shaking (this step may be performed on ice).
3. Stop the reaction by adding 20-30 mL of 1X PBS.
4. Spin the cells (300-400 x g) at 2-8°C and resuspend the pellet in an appropriate buffer for use in the next step of your experimental procedure.
5. Perform a cell count at this time.

Note: In general a small number of residual red cells does not interfere with the proliferation assays and can be gated out from flow cytometric analysis. However, if required, a second round of lysis can be performed.

Lysis of human blood for flow cytometric analysis

When using human whole blood for flow cytometric analysis, the necessary red cell lysing step is incorporated into the staining protocol.

Refer to Best Protocols for the staining protocol.

Bulk lysis of human whole blood

1. Add 10 mL of 1X RBC Lysis Buffer per 1 mL of human blood.
2. Incubate for 10 minutes at room temperature (no more than 15 minutes).
3. Stop the reaction by adding 20-30 mL of 1X PBS.
4. Spin the cells (300-400 x g) at 2-8°C and resuspend the pellet in an appropriate buffer for use in the next step of

your experimental procedure. 5. Perform a cell count at this time.

Note: In general a small number of residual red cells does not interfere with the proliferation assays and can be gated out from flow cytometric analysis. However, if required, a second round of lysis can be performed.

PRODUCT USE LIMITATION

These products are intended for research use only.