

# 1X RBC Lysis Buffer

Catalog Number: 00-4333

For Research Use Only. Not for use in diagnostic procedures.

#### **Product Information**

Contents: 1X RBC Lysis Buffer Catalog Number: 00-4333

**Handling Conditions:** Use within 6 months of opening or by date indicated on the bottle



Formulation: aqueous buffer, no sodium azide Temperature Limitation: Store at 2-8°C

Batch Code: Refer to vial
Use By: Refer to vial

#### Description

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.

## **Applications Reported**

1X RBC Lysis Buffer has been reported for use in flow cytometric analysis and cell culture.

# **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.

#### Lysis of mouse splenocytes:

- 1. Harvest mouse spleen and prepare a single cell suspension.
- 2. Pellet the cells by centrifugation (300-400 x q) 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  $(300-400 \times g)$  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.

Not for further distribution without written consent.

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- 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.

#### **Related Products**

00-4222 Flow Cytometry Staining Buffer 00-4300 10X RBC Lysis Buffer (Multi-species) 00-5333 1-step Fix/Lyse Solution (10X)