1X RBC Lysis Buffer

Catalog Number: 00-4333
GPR: General Purpose Reagents. For Laboratory Use.

Product Information

Contents: 1X RBC Lysis Buffer
REF **Catalog Number:** 00-4333
Handling Conditions: For sterile use, please filter through 0.22 µm membrane.
Temperature Limitation: Store at 2-8°C. Use within 6 months of receipt.
LOT **Batch Code:** Refer to Vial
Use By: Refer to Vial

Description

The eBioscience 1X Red Blood Cell 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. RBC lysis is not necessary when working with mouse thymus and lymph nodes

Applications Reported

Lysis of mouse splenocytes:

1. Harvest mouse spleen and prepare a single cell suspension.
2. Pellet the cells by centrifugation (300-400xg) at 4°C and aspirate the supernatant.
3. Resuspend the pellet in 5 ml/spleen of Lysis Buffer.
4. Incubate at room temperature for 4-5 minutes with occasional shaking (we have performed this step on ice successfully too).
5. Stop the reaction by diluting the Lysis Buffer with 20-30 ml of 1X PBS.
6. Spin the cells (300-400xg) at 4°C and resuspend the pellet in the 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 RBC lysis buffer per 1 ml of mouse blood.
2. Incubate at room temperature for 4-5 minutes with occasional shaking (we have performed this step on ice successfully too).
3. Stop the reaction by diluting the Lysis Buffer with 20-30 ml of 1X PBS.
4. Spin the cells (300-400xg) at 4°C and resuspend the pellet in the 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 peripheral blood for flow cytometric analysis, the necessary red cell lysing step is incorporated into the staining protocol. The staining protocol may be found at: http://www.ebioscience.com/ebioscience/appls/FCS.htm.

Lysis of total human peripheral blood:

1. Add 10 ml of lysis buffer per 1ml of human blood.
2. Incubate for 10 minutes at room temperature (no more than 15 minutes).
3. Stop the reaction by diluting the Lysis Buffer with 20-30 ml of 1X PBS.
4. Spin the cells (300-400xg) at 4°C and resuspend the pellet in the 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.

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