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Red Blood Cell (RBC) Lysis Protocols

Research Use Only

Introduction

Before using peripheral blood or certain lymphoid tissue suspensions for flow cytometric analysis or for in vitro assays, red blood cells (RBC) should be removed. The 1X RBC Lysis Buffer (cat. no. 00-4333) and 10X RBC Lysis Buffer (Multi-species) (cat. no. 00-4300) are formulated for optimal lysis of erythrocytes in single-cell suspensions of human peripheral blood and mouse tissue (such as spleen). The buffers contain ammonium chloride, which lyses RBC with minimal effect on leukocytes. When using human peripheral blood for flow cytometric analysis, the RBC lysing step can be incorporated into the staining protocol. The 1-step Fix/Lyse Solution (10X) (cat. no. 00-5333) is formulated for the combined lysis of RBC and fixation of peripheral blood leukocytes after staining with fluorochrome-conjugated antibodies. All of the RBC lysis reagents are compatible with fluorochrome-conjugated antibodies.

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 (Multi-species) 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

- *Before use, the 10X RBC Lysis Buffer (Multi-species) must be diluted 1:10 with room temperature, reagent-grade water.*
- *The 10X RBC Lysis Buffer (Multi-species) has been shown to work equivalently in blood collected using either heparin or EDTA as the anti-coagulent.*
- *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

- 1X PBS
- 10X RBC Lysis Buffer (Multi-species) (cat. no. 00-4300) or 1X RBC Lysis Buffer (cat. no. 00-4333)
- 50-mL conical tubes
- Flow Cytometry Staining Buffer (cat. no. 00-4222) or other buffer of choice
- 12 x 75 mm round-bottom test tubes
- Primary antibodies (directly conjugated)

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Experimental Procedure

A1. Antibody Staining Followed by Lysis of Whole Peripheral Blood

NOTE: *Refer to bulk lysis protocol (Protocol A2) for RBC lysis before antibody staining.*

1. Aliquot a sample of whole blood into a tube.
   For human, use 100 µl of blood.
   For mouse, use 50-100 µl of blood.
   For rat, use 50-100 µl of blood.
   For canine, use 100 µl of blood.
   For non-human primate, use 100 µl of blood.

2. Add the antibody(s) needed for staining (in a volume no greater than 50 µL) and mix thoroughly. Refer to "Staining Cell Surface Targets for Flow Cytometry Protocols" as found in our Best Protocols.
3. Incubate for 30 minutes in the dark at room temperature.
4. Add 2 mL of room temperature 1X RBC Lysis Buffer, and then pulse vortex or invert to mix.
5. Incubate at room temperature in the dark.
   For human, incubate for 10–15 minutes.
   For mouse, incubate for 4–10 minutes.
   For rat, incubate for 4–10 minutes.
   For canine, incubate for 10–15 minutes.
   For non-human primate, incubate for 10–15 minutes.

NOTE: *Observe turbidity to evaluate red blood cell lysis. Once the sample becomes clear, lysis is complete.*

6. After lysis, centrifuge immediately at 500 *x g* for 5 minutes at room temperature. Decant the supernatant.
7. [Optional] Repeat Steps 4-6.

NOTE: *Step 7 is not typically necessary, as small numbers of residual red blood cells do not interfere with subsequent assays and can be gated out during flow cytometric analysis.*

8. Resuspend cells in 2 mL of Flow Cytometry Staining Buffer and centrifuge as in Step 6.
9. Decant the supernatant and resuspend the cell pellet in an appropriate volume of Flow Cytometry Staining Buffer.
10. Analyze the samples by flow cytometry.

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A2. Bulk Lysis of Human Whole Blood

NOTE: *If cells are to be put in culture, perform all steps using asceptic techniques.*

1. Add 10 mL of 1X RBC Lysis Buffer per 1 mL of human blood.
2. Incubate for 10-15 minutes at room temperature (no more than 15 minutes).

NOTE: *Observe turbidity to evaluate red blood cell lysis. Once the sample becomes clear, lysis is complete.*

3. Centrifuge at 500 *x g* for 5 minutes at room temperature. Decant supernatant.
4. Resuspend the pellet in the appropriate volume of Flow Cytometry Staining Buffer or buffer of choice.
5. Perform a cell count and viability analysis.
6. Proceed with cell staining or culture, as desired.

A3. Lysis of Mouse/Rat Spleen or Bone Marrow Cells

NOTE: *The use of 1X RBC Lysis Buffer (cat. no 00-4333) is recommended for use with mouse and rat tissues.*

NOTE: *If cells are to be put in culture, perform all steps using asceptic techniques.*

1. Harvest tissue and prepare a single-cell suspension. Refer to "Cell Preparation Protocols for Flow Cytometry" found in our Best Protocols.
2. Pellet the cells by centrifugation at 500 *x g* for 5 minutes at room temperature and decant the supernatant.
3. Resuspend the pellet in 3–10 mL of 1X RBC Lysis Buffer.
4. Incubate for 4–5 minutes at room temperature.
5. Stop the lysis reaction by adding 20–30 mL of 1X PBS.
6. Centrifuge immediately at 500 x g for 5 minutes at room temperature. Decant the supernatant.
7. Resuspend cells in 2 mL of Flow Cytometry Staining Buffer or buffer of choice and centrifuge as in Step 6. Decant supernatant.
8. Resuspend cells in an appropriate volume of Flow Cytometry Staining Buffer or buffer of choice.
9. Perform a cell count and viability analysis.
10. Proceed with cell staining or cell culture, as desired.

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Protocol B: Using 1-step Fix/Lyse Solution

The 1-step Fix/Lyse Solution both lyses the RBC and fixes the remaining leukocytes. It is ideal for use when antibody-stained blood samples are to be lysed and fixed before analysis. It may also be used to lyse RBC and fix cells before staining with antibodies; however, it is important to confirm that the antibodies to be used will recognize fixed epitopes on the antigens of interest. Mechanical disruption of lymphoid tissue is generally sufficient to release cells to a single cell suspension.

General Notes

- *Before use, the 1-step Fix/Lyse Solution must be diluted 1:10 with room temperature, reagent-grade water.*
- *The 1-step Fix/Lyse Solution has been shown to work equivalently in blood collected using either heparin or EDTA as the anticoagulant.*

Materials

- 1X PBS
- 1-step Fix/Lyse Solution (10X) (cat. no. 00-5333)
- 50-mL conical tubes
- Flow Cytometry Staining Buffer (cat. no. 00-4222)
- 12 x 75 mm round-bottom test tubes
- Primary antibodies (directly conjugated)

Experimental Procedure

1. To 100 µL of whole blood, add the appropriate antibodies needed for surface staining and mix thoroughly. Refer to "Staining Cell Surface Targets for Flow Cytometry Protocols" as found in our Best Protocols.
2. Incubate for 30 minutes in the dark at room temperature.
3. Add 2 mL of room temperature 1X 1-step Fix/Lyse Solution, then invert gently.
4. Incubate for 15–60 minutes at room temperature in the dark.
5. [Optional] Samples can be stored in 1X 1-step Fix/Lyse Solution for up to 3 days at 2–8ºC in the dark with minimal effect on brightness.

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