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RBC Lysis

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Introduction

Prior to using lymphoid tissue cell suspensions for flow cytometric analysis and/or for *in vitro* functional assays, it is recommended to remove red blood cells (RBCs). The eBioscience 1X RBC Lysis Buffer (Cat. No. 00-4333) or 10X RBC Lysis Buffer (Cat. No. 00-4300) is formulated for optimal lysis of erythrocytes in single cell suspensions of mouse tissues such as spleen and human peripheral blood. The buffer contains ammonium chloride, which lyses red cells with minimal effect on lymphocytes. When using human peripheral blood for flow cytometric analysis, the RBC lysing step can be incorporated into the staining protocol.

General Notes

1. eBioscience offers two solutions for preparing whole blood samples for cell culture or analysis by flow cytometry. Both solutions are provided as sterile.
   - **eBioscience® 10X RBC Lysis Buffer (Multi-species)** and **1X RBC Lysis Buffer** simply lyses the red blood cells in the sample leaving live WBCs cells for analysis.
   - **1-step Fix/Lyse Solution** both lyses the red blood cells and fixes the sample.
2. eBioscience **1-step Fix/Lyse Solution** both lyses the RBCs and fixes the sample. Before using this buffer, you will need to confirm that the antibodies in your staining panel recognize fixed epitopes on the antigens of interest. Please refer to our Antibody Fixation Considerations table online for antibody clone performance following fixation/permeabilization (http://us.ebioscience.com/resources/application/flow-cytometry/clone-performance-after-fix-perm.htm).

Protocol A: 1X or 10X RBC Lysis

Both the 1X and 10X RBC Buffers are designed to lyse RBC in whole blood (*heparin or EDTA collected)* or tissue preparations via using ammonium chloride osmotic shock. The 10X RBC Lysis Buffer (Multi-species) is specially formulated for optimal lysis of peripheral blood. It has been validate to work on whole blood from human, mouse, rat, canine and non-human primate. 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 mouse spleen..

Materials

- 1X PBS
- eBioscience 10X RBC Lysis Buffer (Multi-species) (Cat. No. 00-4300) or 1X RBC Lysis Buffer (Cat. No. 00-4333)
  - Before using, the 10X RBC Lysis Buffer (Multi-species) must be diluted by adding 1 part 10X RBC Lysis Buffer with 9 parts room temperature reagent grade water.

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- 50 mL conical tubes
- Flow Cytometry Staining Buffer (Cat. No. 00-4222)

Experimental Procedure

A. Lysis of mouse/rat spleen or bone marrow cells

1. Harvest tissue and prepare a single-cell suspension.
2. Pellet the cells by centrifugation at 500*xg* for 5 minutes at room temperature and aspirate the supernatant.
3. Resuspend the pellet in 3 - 10 mL of prepared 1X RBC Lysis Buffer.
4. Incubate for 4 - 5 minutes at room temperature.
5. After lysis, stop the reaction by diluting the 1X RBC Lysis Buffer with 20-30 mL of 1X PBS.
6. Centrifuge immediately at 500*xg* for 5 minutes at room temperature. Decant the supernatant.
7. Resuspend the pellet in 2 mL of Flow Cytometry Staining Buffer or buffer of choice and centrifuge again.
8. Decant the supernatant and perform a cell count at this time.
   ***Note**: In general, a small number of residual red cells does not interfere with proliferation assays or can be gated out from flow cytometric analysis. However, if required, a second round of lysis can be performed.*

B. Lysis of mouse or rat blood

1. Add 10 mL of freshly prepared 1X RBC lysis buffer (made from the 10X RBC Lysis Buffer) per 1 mL of *heparin or EDTA* 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. Centrifuge cells at 500x*g* at room temperature 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.*

C. Bulk lysis of human, canine, primate peripheral blood

1. Add 10 mL of prepared 1X RBC Lysis Buffer (made from the 10X RBC Lysis Buffer) per 1 mL of *heparin or EDTA collected* blood.
   ***Note**: If cells are to be put in culture, perform using aseptic techniques.*
2. Incubate for 10-15 minutes at room temperature (no more than 15 minutes).
   ***Note**: Turbidity can be observed to evaluate red blood cell lysis. Once the sample becomes clear, lysis is complete*.
3. [Optional] Stop the reaction by diluting the Lysis Buffer with 20-30 mL of 1X PBS.
4. Centrifuge at 400-600*xg* at room temperature for 4-5 minutes.
5. Decant the supernatant. If Step 3 was performed, skip to Step 7.

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6. If reaction was not stopped as stated in Step 3, wash cells with 50 mL PBS or Flow Cytometry Staining Buffer. Centrifuge at 400-600*xg* at room temperature for 4-5 minutes.
7. Resuspend the pellet in the appropriate buffer for use in the next step of your experimental procedure.
8. Perform a cell count at this time.
   Note: *In general a small number of residual red cells does not interfere with the proliferation and can be gated out from subsequent flow cytometric analysis. However, if required, a second round of lysis can be performed.*

D. Whole Blood Antibody Staining followed by RBC lysis in flow tubes

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.
   Note*: The 10X RBC Lysis buffer (Multi-species) has been shown to work equivalently in blood collected in either heparin or EDTA as the anti-coagulant.*
2. Add the antibody(s) needed for staining (in a volume no greater than 50 µl) and mix thoroughly.
3. Incubate for 30 minutes in the dark (if staining fluorochrome-conjugated antibodies) at room temperature.
4. Add 2 mL of room temperature prepared 1X RBC Lysis Buffer (made from the 10X 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: *Turbidity can be observed to evaluate red blood cell lysis. Once the sample becomes clear, lysis is complete.*
6. After lysis, centrifuge immediately at 500*xg* for 4-5 minutes at room temperature. Decant the supernatant.
7. [Optional] The samples can again be incubated with additional 1X RBC Lysis buffer (1 mL for 3 minutes) if further removal of red blood cells is needed. However, this step is not typically necessary since small numbers of residual red blood cells do not interfere with subsequent assays and can be gated out during flow cytometric analysis.
8. Resuspend the pellet in 2 mL of Flow Cytometry Staining Buffer and centrifuge again.
9. Decant the supernatant and resuspend the cell pellet in approximately 200 µL of Flow Cytometry Staining Buffer.
10. Analyze the samples by flow cytometry.

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Protocol B: 1-step Fix/Lyse Solution

The eBioscience 1-step Fix/Lyse Solution enables lysis of red blood cells after staining peripheral blood cells with fluorochrome conjugated antibodies. This solution has been specially formulated to lyse non-nucleated erythrocytes while maintaining a fixed and labeled leukocyte population. Therefore, whole blood samples can be stained for the appropriate markers, RBC-lysed, washed, and then analyzed by flow cytometry. The eBioscience 1-Step Fix/Lyse Solution is compatible with antibodies conjugated to organic dyes, eFluor® nanocrystals, and tandem dyes.

Materials

- 1X PBS
- eBioscience 1-Step Fix/Lyse buffer (10X) (Cat. No. 00-5333)
  - Before using, eBioscience 1-step Fix/Lyse Solution (10X) must be diluted to 1X with room temperature reagent grade water.
- 50 mL conical tubes
- Flow Cytometry Staining Buffer (Cat. No. 00-4222)

Experimental Procedure

A. Whole Blood Antibody Staining followed by lysis with 1-Step Fix/Lyse Buffer

1. To 100 µL of whole blood, add the appropriate antibodies needed for surface staining and mix thoroughly. Please refer to Staining Cell Surface Antigens for Flow Cytometry Protocols (Protocol B) found in our Best Protocols section.
   ***Note**: eBioscience 1-step Fix/Lyse Solution has been shown to work equivalently in blood collected with either heparin or ETDA as the anticoagulant.*
2. Incubate for 30 minutes in the dark at room temperature.
3. Add 2 mL of room temperature 1X eBioscience 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 buffer for up to 3 days at 4^{o}C in the dark with minimal effect on brightness. eBioscience tandem-dye conjugated antibodies are also quite stable under these storage conditions. However, for optimal compensation, we do recommend having single color stained cells stored under the same conditions to set compensation.
6. Centrifuge at 500*xg* for 5 minutes at room temperature. Decant the supernatant.
7. Wash once with 2 mL Flow Cytometry Staining Buffer and spin again. Decant the supernatant.
8. Resuspend the cell pellet in 200 µL Flow Cytometry Staining Buffer.
9. Analyze samples by flow cytometry.
   ***Note**: Intracellular markers can be stained after Step 5 (Fix/Lyse) but will require permeabilization. It is important to make sure the specific antibody will work after this type of fixation. For example antibodies to transcription factors such as Foxp3 will not work in this buffer/solution system.*

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