Introduction:

BioLegend’s Red Blood Cell (RBC) Lysis Buffer (Cat. No. 420301) has been designed, formulated, and tested to ensure optimal lysis of RBCs in single cell suspensions of hematopoietic tissues, such as spleen, as well as RBCs derived from peripheral blood. RBC Lysis Buffer contains ammonium chloride, which has been demonstrated to lyse RBCs with a minimum of harmful effects on lymphocytes.

Method I: Lysis of Mouse Spleen RBCs

1. Harvest mouse spleen and prepare a single cell suspension.
2. Pellet the cells by centrifugation (350 x g); aspirate the supernatant.
3. Dilute the 10X Red Blood Cell Lysis Buffer to 1X working concentration with deionized water and resuspend the pellet in 3-5 ml of 1X Lysis Buffer.
4. Incubate on ice for 4-5 minutes with occasional shaking.
5. Stop the reaction by diluting the Lysis Buffer with 10-20 ml of 1X PBS.
6. Spin the cells (350 x g), discard the supernatant, and resuspend the pellet in the appropriate buffer (e.g., BioLegend Cell Staining Buffer Cat. No. 420201).
7. Count cells, adjust density, and proceed with cell staining procedures.

Method II: Lysis of Human Peripheral Blood RBCs:

1. Dilute the 10X RBC Lysis Buffer to 1X working concentration with deionized water. Warm the 1X solution to room temperature prior to use.
2. Add 2.0 ml of 1X RBC Lysis Buffer to each tube containing up to 100 µl of whole blood.
3. Gently vortex each tube immediately after adding the lysing solution. Incubate at room temperature, protected from light, for 10-15 minutes.
4. Centrifuge 350 x g for 5 minutes. Aspirate supernatant without disturbing pellet, and resuspend the pellet in the appropriate buffer (e.g., BioLegend Cell Staining Buffer, Cat. No. 420201).

References:


Reagent List:

1. Cell Staining Buffer (Cat. No. 420201)
2. RBC Lysis Buffer (Cat. No. 420301)