Lysing red blood cells

Lysing and blood prep protocols can be found here

Lysing Solution recipe

Ammonium chloride lyse (10X concentration)
NH4Cl (ammonium chloride) 8.02gm
NaHCO3 (sodium bicarbonate) 0.84gm
EDTA (disodium) 0.37gm
QS to 100ml with Millipore water. Store at 4C for six months.

Working solution
Dilute 10ml 10X concentrate with 90 ml water. Refrigerate until use.

Bulk Lysis of human whole blood

Note: If cells are to be put in culture, perform all steps using aseptic 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

L**ysis 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 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.