10X RBC Easy-Lyse Buffer (Mouse and Human Optimized)
Flow Cytometry Support Tools

Prod. No.: R1363
Lot No.: Refer to Lot Specific Datasheet for more information
Pkg. Size: 100 ml, Custom
Storage: 2-8°C Detailed storage instructions below.

Description
Leinco Technologies Red Blood Cell (RBC) Easy-Lysis Buffer has been designed, formulated, and tested to gently lyse RBCs in single cell suspensions while maintaining exquisite population of leukocytes for multiple species. This RBC Lysis Buffer is supplied as a 10X solution containing ammonium chloride and EDTA (Also contains Proprietary buffered salt formulation), and should be diluted in deionized water prior to use. NOTE: Nucleated RBCs are not effectively lysed with ammonium chloride.

Working Dilutions
This RBC Easy-Lyse reagent is provided as a 10X concentrates for easy dilution using distilled water. Dilute 1 ml of desired reagent with 9 ml of distilled water or an equivalent scaled-up dilution depending on amounts needed. The pH of the 1X solution should fall within the rage of pH 7.1-7.4. Adjust the pH if necessary and warm the 1X solution to room temperature prior to use.

Directions For Use With Directly Labeled Antibodies

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 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 20-30 ml of 1X PBS.
6.) Spin the cells (350 x g) and discard the supernatant.
7.) Resuspend the pellet in the appropriate buffer
8.) Count cells, adjust density, and proceed with cell staining procedures.

Lysis pf Human Peripheral Blood RBSs (Consider alternative Leinco Part Number K104)

1.) Dilute the 10X RBC Easy-Lyse Buffer to 1X working concentration with deionized water. Warm the 1X solution to room temperature prior to use.

2) . Add 2.0 ml og RBC Lyse Buffer to each tube containing 100 µl of whole blood with directly labeled antibody according to manufacturer’s recommended procedures.

3.) Gently vortex the blood immediately after adding 2 ml of the working concentration 1X RBC Lyse buffer and again vortex the cells vigorously. It is important to vortex each tube immediately after adding RBC Lyse solution.

Products are for research use only. Not for use in diagnostic or therapeutic procedures.
4.) Incubate cells at room temperature for ten minutes. Exposure to RBC Easy-Lyte should not exceed 20 minutes.

5.) Centrifuge leukocytes for 5 minutes at 300 - 500 x g. Decant or Aspirate the supernatant and wash the cells with 2 ml of the working concentration of 1 X washing buffer or an appropriate buffer by gently vortexing the cells and centrifuging for 5 minutes at 300 - 500 x g. If flow cytometric analysis is to be performed within an hour and no fixing is desired, resuspend the cells by gently vortexing in 0.5 ml of wash buffer. Cells are now ready for analysis.

6.) If analysis is to be performed after one hour or if fixing is desired, add 0.5 ml of the working concentration 1X fixative solution to the pelleted cells from step 4. Vortex the cells gently and they are now ready for flow cytometric analysis. Do not fix cells if they are to be used for tissue culture. Fixed cells should be stored at 4°C and are now stable up to 48 hours for analysis.

Country of Origin
USA