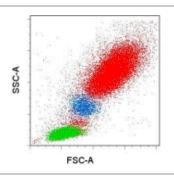
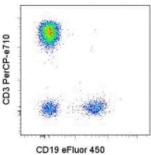


# 1-step Fix/Lyse Solution (10X)

Catalog Number: 00-5333

GPR: General Purpose Reagents. For Laboratory Use.





Normal human peripheral blood cells were stained with Anti-Human CD45 FITC (cat. 11-9459), Anti-Human CD3 PerCP-eFluor® 710 (cat. 46-0037), and Anti-Human CD19 eFluor® 450 (cat. 48-0199), and then incubated with eBioscience 1-step Fix/Lyse Solution (10X) that was diluted to 1X with water for 15 minutes at room temperature. Cells were spun, washed once in flow stain buffer, and then analyzed. (left) CD45+ granulocytes (red), monocytes (blue), and lymphocytes (green) can be seen in the forward vs. side scatter plot of total viable cells. (right) Analysis of CD45+ lymphocytes.

#### **Product Information**

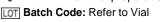
Contents: 1-step Fix/Lyse Solution (10X)

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X

Temperature Limitation: Store at 2-8°C. Use the 1X solution

within 1 month of preparation.



Use By: Refer to Vial

Contains 10% formaldehyde and 30% diethylene glycol

### Description

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. Isolation of mononuclear cells by gradient centrifugation (e.g. Ficoll-Paque density gradient separation) is not required when using this solution. The eBioscience 1-Step Fix/Lyse Solution is compatible with antibodies conjugated to organic dyes, eFluor® nanocrystals, and tandem dyes.

### **Applications Reported**

The eBioscience 1-step Fix/Lyse Solution has been reported for use in flow cytometric analysis.

### **Applications Tested**

The eBioscience 1-Step Fix/Lyse Solution has been tested by flow cytometric analysis of normal human, mouse, rat, and non-human primate (rhesus macaque) peripheral blood cells.

## **Related Products**

11-9459 Anti-Human CD45 FITC (2D1)

46-0037 Anti-Human CD3 PerCP-eFluor® 710 (OKT3)

48-0199 Anti-Human CD19 eFluor® 450 (HIB19)

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## eBioscience 1-Step Fix/Lyse Solution (10X)

## **Research Use Only**

## Protocol: Staining and lysing with eBioscience 1-step Fix/Lyse Solution (10X)

### **Experimental Procedure**

Before using, eBioscience 1-step Fix/Lyse Solution (10X) must be diluted to 1X with room temperature reagent grade water.

1. To 100  $\mu$ L of whole blood, add the appropriate antibodies needed for staining and mix thoroughly.

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 min in the dark at room temperature.
- 3. Add 2 mL of room temperature 1X eBioscience 1-step Fix/Lyse Solution, then vortex or invert gently.
- 4. Incubate for 15 to 60 min at room temperature in the dark.
- 5. Optional: Samples can be stored in buffer for up to 48 hours with minimal effect on brightness. Tandems are also quite stable, but we recommend having single color stained cells to set compensation.
- 6. Centrifuge at  $500 \times g$  for 5 min at room temperature. Decant the supernatant.
- 7. Wash 1X with 2 mL flow stain buffer and spin again. Decant the supernatant.
- 8. Resuspend the cell pellet in 200 µL flow stain buffer.
- 9. Analyze samples by flow cytometry.

### Additional 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. Please refer to the Foxp3 Whole Blood Staining Kit (Cat. No. 88-8996).