For Research Use Only

Intracellular Flow Cytometry Fixation & Permeabilization Buffer Kit

Catalog Number: PF00019

www.ptglab.com

Product Information

Proteintech's Intracellular Flow Cytometry Fixation & Permeabilization Buffer Kit (Catalog No. PF00019) is suitable for flow cytometry analysis of intracellular protein targets as well as cytokine and chemokine targets. The kit contains buffers for the fixation and permeabilization of cells.
This product contains formaldehyde, FBS, and sodium azide (≤ 0.09%).

Components

Component	Volume
Fix/Perm Buffer PF00019-A	125 mL
Perm/Wash Buffer (10X) PF00019-B	100 mL

Package

1 kit

Storage

Store at 4 degrees C for up to 6 months.

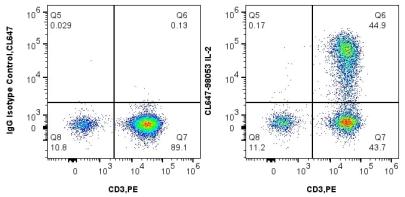
Usage

It is recommended that cells are stained with a fixable viability dye such as a Phantom Dye Viability Dye, and for any surface markers prior to fixation.
1. Dilute 1 part Perm/Wash Buffer (10X) (PF00019-B) with 9 parts deionized water to make Perm/Wash Buffer (1X). Around 7 mL Perm/Wash Buffer (1X) are needed per tube of 1x10^6 cells or 2mL per well in a 96-well plate.
2. Wash cells with PBS. Add 500 uL Fixation Buffer (PF00019-A) per tube or 200 uL Fixation Buffer per well. Mix and incubate at RT in the dark for 15 minutes. Centrifuge at 400-600 g for 5 minutes. Aspirate the supernatant.
3. Wash the cells: add 1 mL Perm/Wash Buffer (1X) per tube or 200 uL per well in a 96-well plate. Mix well by pipetting. Centrifuge at 400-600 g for 5 minutes. Aspirate the supernatant. Repeat.
4. Resuspend cells in 100 uL Perm/Wash Buffer (1X).
5. Stain cells with primary antibodies at the optimal titration according to vendor recommendations.
6. After incubation with primary antibody, wash cells twice as in step 3.
7. If staining with secondary antibody, continue to the next step. If not, skip to step 10.
8. Dilute secondary antibody to the optimal titration with Perm/Wash Buffer (1X). Add 100 uL diluted secondary antibody to the cells.
9. After incubation, wash cells twice as in step 3.
10. Resuspend cells in 200 uL Perm/Wash Buffer (1X). and acquire samples on a flow cytometer.

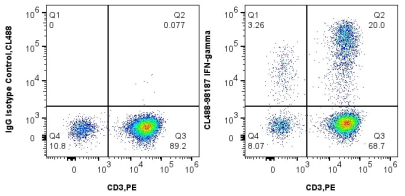
Notes

1. This product contains formaldehyde and sodium azide. Take protective measures and avoid contact with eyes and skin.
2. During incubation, ensure cells are fully suspended in buffer and avoid cells sticking to the sides of the tube. This will allow cells to be fully permeabilized and prevent false negative populations.

Validation Data



1x10^6 PMA, Ionomycin and Brefeldin A treated human PBMCs were intracellularly stained with 5 uL CoraLite® Plus 647 Anti-Human IL-2 Rabbit Recombinant Antibody (CL647-98053, Clone: 240416E3) or 5 uL CoraLite® Plus 647 Rabbit IgG Isotype Control Recombinant Antibody (CL647-98136, Clone: 240953C9) and 5 uL PE Anti-Human... CD3 (OKT3) Mouse IgG2a Recombinant Antibody (PE-65569, Clone: OKT3). Cells were fixed and permeabilized with Intracellular Flow Cytometry Fixation & Permeabilization Buffer Kit (PF00019).



1x10^6 PMA, Ionomycin and Brefeldin A treated human PBMCs were intracellularly stained with 5 uL CoraLite® Plus 488 Anti-Human IFN-gamma Rabbit Recombinant Antibody (CL488-98187, Clone: 241390B10) or 5 uL CoraLite® Plus 488 Rabbit IgG Isotype Control Recombinant Antibody (CL488-98136, Clone: 240953C9) and 5uL PE Anti-Human... CD3 (OKT3) Mouse IgG2a Recombinant Antibody (PE-65569, Clone: OKT3). Cells were fixed and permeabilized with Intracellular Flow Cytometry Fixation & Permeabilization Buffer Kit (PF00019)