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Intracellular Permeabilization Buffer (10X)

Reagent for the permeabilization of fixed cells

Catalog #100-1451 150 mL

Product Description

Intracellular Permeabilization Buffer (10X) is a concentrated buffer recommended for the permeabilization of cells treated with Fixation Buffer (Catalog #100-1450) or a fixative of choice prior to immunostaining intracellular cytokines and other cytoplasmic antigens. This buffer maintains cell membrane permeability throughout staining and washing steps, and allows for optimal resolution and low background when performing flow cytometry analysis of fluorochrome-labeled cells.

Properties

Stability and Storage: Stable until expiry date (EXP) on label. Store at 2 - 8°C.

Contains:

- Sodium azide (< 1%)

Directions for Use

1. Prepare 1X Intracellular Permeabilization Buffer by diluting 1 in 10 in distilled water.
2. Prepare a single-cell suspension of cells and aliquot into flow cytometry tubes or wells of a 96-well plate.
3. Optional: Stain cell surface antigens with fluorochrome-conjugated antibodies as directed in the protocol of choice.
   NOTE: It is recommended to label cells with a fixable viability dye (e.g. GloCell™ Fixable Viability Dye Red 780 [Catalog #75007.1]) and cell surface markers prior to fixation and permeabilization.
4. Wash cells with 1 - 2 mL/tube or 100 - 200 µL/well of Dulbecco's Phosphate Buffered Saline with 2% Fetal Bovine Serum (Catalog #07905) or a staining buffer of choice.
5. Centrifuge cells at 300 - 400 x g for 5 minutes at room temperature (15 - 25°C). Remove and discard the supernatant.
6. Resuspend cells in 100 µL of Fixation Buffer (Catalog #100-1450) or fixative of choice per tube or well.
   NOTE: It is recommended that Fixation Buffer be titrated for optimal performance for each application. Fixation Buffer may be diluted with D-PBS (e.g. Catalog #37350).
7. Incubate at room temperature for 20 - 30 minutes. Protect from light.
8. Wash cells with 1 - 2 mL/tube or 100 - 200 µL/well of PBS with 2% Fetal Bovine Serum or a staining buffer of choice.
9. Centrifuge at 300 - 400 x g for 5 minutes at room temperature. Remove and discard the supernatant.
10. Resuspend cells in 1 - 2 mL/tube or 100 - 200 µL/well of 1X Intracellular Permeabilization Buffer.
11. Incubate at room temperature for 5 minutes. Protect from light.
12. Centrifuge at 300 - 400 x g for 5 minutes at room temperature. Remove and discard the supernatant.
13. Resuspend the cells in 50 - 100 µL/well of 1X Permeabilization Buffer per tube or well. Add fluorochrome-conjugated primary antibodies for intracellular antigens, as directed in the protocol of choice. We recommend pre-determining the optimal concentration of antibody to use by

titration.
NOTE: Always prepare antibodies for intracellular staining in 1X Permeabilization Buffer.

14. Incubate at room temperature for 20 - 60 minutes. Protect from light.
15. Wash cells with 1 - 2 mL/tube or 100 - 200 µL/well of 1X Intracellular Permeabilization Buffer.
16. Centrifuge at 300 - 400 x g for 5 minutes at room temperature. Remove and discard the supernatant.
17. Wash cells with 1 - 2 mL/tube or 100 - 200 µL/well of PBS with 2% Fetal Bovine Serum or a staining buffer of choice.
18. Centrifuge at 300 - 400 x g for 5 minutes at room temperature. Remove and discard the supernatant.
19. Resuspend labeled cells in an appropriate volume of staining buffer. Cells are now ready for analysis by flow cytometry.