TECHNICAL DATA SHEET

Flow Cytometry Perm Buffer (10X)

Catalog Number: TNB-1213-L150

PRODUCT INFORMATION

Contents: Flow Cytometry Perm Buffer (10X)

Use By: 6 months from date of receipt

Storage Conditions: 2-8°C

Formulation: Flow Cytometry Perm Buffer (10X) contains sodium azide. Use appropriate personal protective equipment in order to avoid contact with skin and eyes.

DESCRIPTION

Tonbo Biosciences Flow Cytometry Perm Buffer (10X) is provided as a concentrate which, when diluted with distilled water to a 1X solution, provides best results in intracellular staining protocols for cytokines and other cytoplasmic antigens, by maintaining membrane permeabilization throughout staining and wash steps. The Flow Cytometry Perm Buffer is formulated for optimal resolution and low background when used in fluorophore-labeled antibody staining protocols, followed by flow cytometric analysis.

PREPARATION

Flow Cytometry Perm Buffer is supplied as a 10X stock solution and must be diluted to a 1X solution with distilled water prior to use.

APPLICATION NOTES

Flow Cytometry Perm Buffer is intended for use as a wash and incubation buffer for intracellular staining protocols. The 1X working solution should be used for all washing and staining steps subsequent to cell surface staining and fixation.

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Intracellular Cytokine Staining Protocol

Flow Cytometry Perm Buffer (10X) Cat. No. TNB-1213-L150

Other Materials Required:

- Flow Cytometry Staining Buffer (Stain Buffer) (1X PBS with 2% FBS, 0.09% Na-Azide)
- 4% Paraformaldehyde Fixation Buffer (Fix Buffer) – use a methanol-free formulation

Buffer and Solution Preparation

Prepare a 1X working solution of Permeabilization Buffer by diluting the 10X concentrate in distilled water prior to use.

Stimulation of Cells (optional)

A variety of methods can be used to stimulate cells to produce cytokines and investigators should determine which protocols will allow for appropriate activation of their particular sample to meet experimental needs. Tonbo Biosciences offers a **Cell Stimulation Cocktail (TNB-4975-UL100)** that can be used to elicit cytokine production and retention in a variety of cell types allowing for detection of intracellular proteins by flow cytometry. Refer to our '*Cell Stimulation for Cytokine Production Protocol*' for further details.

Experimental Procedure

1. Aliquot cell samples to tubes in a volume and at a cell concentration suitable for staining.
2. Stain cell surface antigen(s) with the recommended optimal concentration of fluorochrome labeled antibodies.
3. Wash cells in 1-2 mL Stain Buffer. Centrifuge at 300-400 x *g* for 5 minutes at room temperature and discard supernatant.

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4. Approximately 100 μL residual volume will generally remain in the tube. Vortex tube (<5 seconds) to dissociate the cell pellet.
5. Fix cells by adding 100 μL of Fix Buffer and vortex (< 5 seconds).
6. Incubate tubes in the dark at room temperature for 20-60 minutes.
7. Wash cells in Stain Buffer. Centrifuge at 300-400 x *g* for 5 minutes at room temperature and discard supernatant.
8. Resuspend the cell pellet in 2 mL of 1X Permeabilization Buffer.
9. Incubate tubes in the dark at room temperature for 5 minutes.
10. Centrifuge samples at 300-400 x *g* for 5 minutes at room temperature room temperature for 5 minutes and discard the supernatant.
11. Resuspend the cells in 100 μL of 1X Permeabilization Buffer. Add the recommended amount of fluorochrome-labeled antibody for detection of intracellular antigen(s) to cells and incubate in the dark at room temperature for 20-60 minutes.
    Note: **Antibodies for intracellular staining should always be prepared in 1X Permeabilization Buffer**.
12. Add 2 mL of 1X Permeabilization Buffer to each tube.
13. Centrifuge samples at 300-400 x *g* for 5 minutes at room temperature and discard the supernatant.
14. Add 2 mL of Stain Buffer to each tube.
15. Centrifuge samples as in Step 13 and discard the supernatant.
16. Resuspend stained cells in an appropriate volume of Stain Buffer and acquire samples on a flow cytometer.

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