Cyto-Fast™ Fix/Perm Buffer Set

Catalog# / Size	426803 / 300 tests
Regulatory Status	RUO
Other Names	CytoFast
Description	Cyto-Fast™ Fix/Perm Buffer Set is composed of Cyto-Fast™ Fix Perm Solution and Cyto-Fast™ Perm Wash Solution (10X). It is designed for fixation and permeabilization of mammalian cells for intracellular staining such as cytokines and other cytoplasmic molecules.

Product Details

Storage & Handling

Upon receipt, store at 2-8°C.

Application

ICFC - *Quality tested*

Recommended Usage

Procedure for staining in round-bottom 5mL tubes:

1. Dilute the Cyto-Fast™ Perm Wash solution (10X) to 1X using deionized water
2. Aliquot 100 µl of cells (2 x 10^5-1 x 10^6) of interest into a 12 x 75 mm tube
3. Add 150 µl of Cyto-Fast™ Fix/Perm Buffer and mix
4. Incubate for 20 minutes at room temperature
5. Add 1 ml of 1X Cyto-Fast™ Perm Wash solution
6. Centrifuge at 350 xg for 5 minutes, discard supernatant
7. Repeat steps 5 - 6
8. Stain cells with optimal concentration of intracellular antibodies. Prepare antibodies in 1X Cyto-Fast™ Perm Wash Solution, in 100 µl total volume
9. Incubate 20 minutes at room temperature in the dark
10. Wash cells with 1 ml of 1x Cyto-Fast™ Perm Wash solution
11. Centrifuge at 350 xg for 5 minutes, discard supernatant
12. Add 1 mL of Cell Staining Buffer (Cat. No. 420201)
13. Centrifuge at 350 xg for 5 minutes, discard supernatant
14. Resuspend the cells in 300 µl of Cell Staining Buffer
15. Acquire samples on a flow cytometer.

Procedure for staining in 96-well U-bottom plates:

1. Dilute the Cyto-Fast™ Perm Wash solution (10X) to 1X using deionized water
2. Aliquot 100 µl of cells (2 x 10^5-1 x 10^6) of interest into individual wells in a 96-well U-bottom plate
3. Centrifuge at 350 xg for 5 minutes, discard supernatant, and loosen cell pellet.
4. Add 100 µl of Cyto-Fast™ Fix/Perm Buffer and mix
5. Incubate for 20 minutes at room temperature
6. Add 100 µl of 1X Cyto-Fast™ Perm Wash solution
7. Centrifuge at 350 xg for 5 minutes, discard supernatant
8. Repeat steps 5 – 6 by adding 200 µl of 1X Cyto-Fast™ Perm Wash solution
9. Stain cells with optimal concentration of intracellular antibodies. Prepare antibodies in 1X Cyto-Fast™ Perm Wash Solution, in 100 µl total volume
10. Incubate 20 minutes at room temperature in the dark
11. Wash cells by adding 100 µl of 1x Cyto-Fast™ Perm Wash solution
12. Centrifuge at 350 xg for 5 minutes, discard supernatant
13. Add 200 µl of Cell Staining Buffer (Cat. No. 420201)
14. Centrifuge at 350 xg for 5 minutes, discard supernatant
15. Resuspend the cells in 200 µl of Cell Staining Buffer
16. Acquire samples on a flow cytometer.

Addional Notes

- Cells can be bulk fixed. To bulk fix the cells, add 1.5 ml of Cyto-Fast™ Fix/Perm Buffer for each 1 x 10^7 cells and incubate for 15-20 minutes at room temperature.
- Staining for surface markers can be performed prior to fix/perm. Follow the Cell Surface Flow Cytometry Staining Protocol. Proceed to follow the Cyto-Fast™ Fix/Perm Buffer Set protocol outlined above after primary (and/or secondary) surface antibody staining steps.
- If cells are to be immediately stained post-fix/perm, wash cells 2 x with 10 ml of 1X Cyto-Fast™ Perm/Wash. Aliquot 100µl of cells (2 x 10^5-1 x 10^6) of interest into a 12 x 75 mm tube and stain cells with optimal concentration of antibodies (continue with procedure listed above at step 9).
- If cells are to be stained at a later time post-fix/perm, wash cells with 10 ml of Cell Staining

Buffer. Cells can be stored for up to one week in Cyto-Last™ Buffer (Cat. No. 422501).

- To stain cryopreserved cells, quickly thaw the cells by placing them in a 37°C water bath, wash with 10 ml of 1X Cyto-Fast™ Perm/Wash Solution and centrifuge for 5 minutes at 200-300xg, discard supernatant. Re-suspend cells in 1X Cyto-Fast™ Perm/Wash for 15-20 minutes prior to staining.

Application Notes

Cyto-Fast™ Fix/Perm Buffer Set Contents:

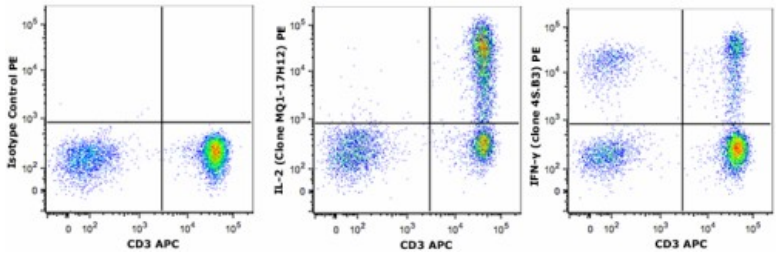
- Cyto-Fast™ Fix/Perm Solution (50 mL)
- Cyto-Fast™ Perm Wash Solution 10X (100 mL)

Antigen Details

Molecular Family Cytokines/Chemokines

Gene ID NA

Product Data



PMA + ionomycin stimulated (3 hours) human peripheral blood lymphocytes (in the presence of monensin) were fixed and permeabilized with Cyto-Fast™ Fix/Perm Buffer Set. Cells were then stained with CD3 (UCHT1) APC and isotype control PE (left panel), IL-2 (clone MQ1-17H12) PE (middle panel), or IFN-γ (clone 4S.B3) PE (right panel).

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