

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 (2×10^5-1×10^6) of interest into a 12 x 75 mm tube 3. Add 150 µL of Cyto-Fast™ Fix/Perm Solution 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 (2×10^5-1×10^6) 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 Solution 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 6 - 7 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.

Additional Notes

- Cells can be bulk fixed. To bulk fix the cells, add 1.5 mL of Cyto-Fast™ Fix/Perm Solution for each 1×10^7 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 Solution. Aliquot 100µL of cells (2×10^5 - 1×10^6) 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 Solution for 15-20 minutes prior to staining.
- The Cyto-Fast™ Fix/Perm Buffer set (10x) may have crystallization or precipitation when it is stored at 2-8°C this is normal and does not affect the buffer performance. If heavy precipitation is observed after diluting to a 1X working solution, it can be filtered to clarify the solution.

Application Notes

Cyto-Fast™ Fix/Perm Buffer Set Contents:

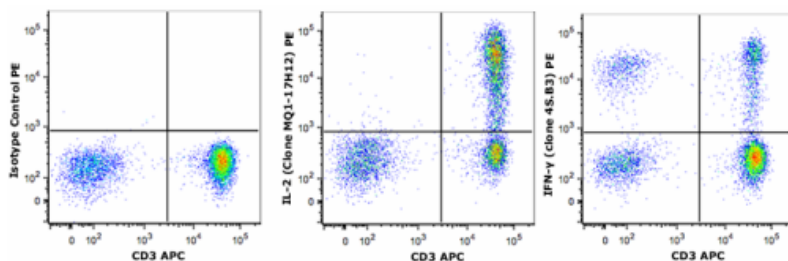
- Cyto-Fast™ Fix/Perm Solution (50 mL)
- Cyto-Fast™ Perm Wash Solution 10X (100 mL)

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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