

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 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 (2×10^5 - 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 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.

Additional Notes

- Cells can be bulk fixed. To bulk fix the cells, add 1.5 ml of Cyto-Fast™ Fix/Perm Buffer 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. 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 for 15-20 minutes prior to staining.

Application Notes

Cyto-Fast™ Fix/Perm Buffer Set Contents:

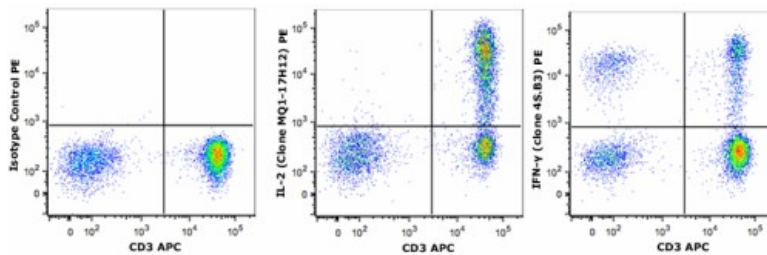
- Cyto-Fast™ Fix/Perm Solution (50 mL)
- Cyto-Fast™ Perm Wash Solution 10X (100 mL)

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).

For research use only. Not for diagnostic use. Not for resale. BioLegend will not be held responsible for patent infringement or other violations that may occur with the use of our products.

*These products may be covered by one or more Limited Use Label Licenses (see the BioLegend Catalog or our website, www.biolegend.com/ordering#license). BioLegend products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products, reverse engineer functionally similar materials, or to provide a service to third parties without written approval of BioLegend. By use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses. Unless otherwise indicated, these products are for research use only and are not intended for human or animal diagnostic, therapeutic or commercial use.

8999 BioLegend Way, San Diego, CA 92121 www.biolegend.com
Toll-Free Phone: 1-877-Bio-Legend (246-5343) Phone: (858) 768-5800 Fax: (877) 455-9587