Technical Data Sheet

Fixation Buffer

Product Information

Material Number: **554655**
Size: 100 mL

Description

BD Cytofix™ Fixation Buffer is intended to preserve human and rodent lymphoid cells for the subsequent immunofluorescent staining of intracellular cytokines. BD Cytofix can also be used to preserve the light-scattering characteristics and fluorescence intensities of human and rodent hematopoietic cells that have been stained by immunofluorescence for subsequent flow cytometric analysis.

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Recommended Assay Procedure:

BD Cytofix™ Fixation Buffer can be used to fix unstained cells for subsequent immunofluorescent staining of intracellular cytokines. The suitability of fixing cells for immunofluorescent staining depends on whether the fluorescent antibodies can specifically detect their cognate antigens in a fixed form. With respect to intracellular cytokines, BD Biosciences offers a large panel of conjugated anti-cytokine antibodies that can be successfully used to stain fixed and permeabilized cells. For the staining of antigens expressed on the surface of fixed cells, several fluorescent antibodies directed against mouse cell surface antigens have been identified to be useful.

BD Cytofix can also be used to fix cells after immunofluorescent staining in order to preserve the light-scattering signals and fluorescent intensities of cells for analysis at a later time. Cell Fixation Buffer may be useful to avoid the capping or shedding of fluorescent antibodies and/or surface antigens during the period before flow cytometric analysis.

Procedure for fixing cells with BD Cytofix™:

1. Pellet 10^6 suspended cells (e.g., cytokine-producing cells generated by stimulatory culture) by centrifugation (250 - 300 x g) and carefully remove supernatants to avoid cell loss.
2. Add either 200 µl (for microwell plates) or 500 µl (for tubes) aliquots of cold DPBS containing protein and NaN3, gently resuspend cells, pellet, and remove supernatants.
3. Repeat step 2.
4. Add either 100 µl (for microwell plates) or 250 µl (for tubes) aliquots of fixation buffer to each cell pellet and resuspend the cells by either pipetting or vortexing. Incubate the cells with fixation buffer for 15 to 30 min at 4°C. (Cell aggregation can be avoided by vortexing prior to the addition of the fixation buffer.)
5. Fixed cells should be washed and suspended in a buffer that contains protein and NaN3, e.g., either Stain Buffer (FCS) [Cat. No. 554656] or Stain Buffer (BSA) [Cat. No. 554657]. Store the fixed cells at 4°C (protected from light) for subsequent immunofluorescent staining of intracellular cytokines. It is recommended that fixed cell samples be read as soon as possible, i.e., within one week.

For the immunofluorescent staining of intracellular cytokines, cells that have been previously fixed with BD Cytofix™ can be washed two times in a buffer that contains protein and NaN3 followed by incubating the cells for at least 10 minutes (4°C) in a buffer containing the cell-permeabilizing agent, saponin. BD Perm/Wash™ buffer (Cat. No. 554723) is ideally suited for this purpose. The fixed and permeabilized cells can then be stained for intracellular cytokines.

Procedure for fixing immunofluorescently-stained cells with BD Cytofix™:

Cells stained by immunofluorescence for cell surface antigens can be fixed as described above and stored (4°C, protected from light) for subsequent analysis by flow cytometry (or fluorescence microscopy).

Note: BD Cytofix/Cytoperm™ solution (Cat. No. 554722) and the BD Perm/Wash™ buffer (Cat. No. 554723) are included in BD Cytofix/Cytoperm Kit (Cat. No. 554714) as well as the BD Cytofix/Cytoperm Plus Kit with GolgiStop™ (containing monensin; Cat. No. 554715) and BD Cytofix/Cytoperm Plus Kit with GolgiPlug™ (containing brefeldin A; Cat. No. 555028).

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Danger: *BD Cytofix™ Fixation Buffer contains 4.21% formaldehyde (w/w).*

Hazard statements

Harmful if inhaled.
Causes skin irritation.
Causes serious eye damage.
May cause an allergic skin reaction.
Suspected of causing genetic defects.
May cause cancer. Route of exposure: Inhalative.

Precautionary statements

Obtain special instructions before use. Do not handle until all safety precautions have been read and understood.
Wear protective clothing / eye protection/ gloves.
Contaminated work clothing should not be allowed out of the workplace. Wasth contaminated clothing before reuse.
Do not breathe mist/vapours/spray.
IF exposed or concerned: Get medical advice/attention.
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
If skin irritation or rash occurs: Get medical advice/attention.

Storage Statement

Store locked up.

Disposal Statement

Dispose of contents/container to an appropriate treatment and disposal facility in accordance with applicable laws and regulations, and product characteristics at time of disposal.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
554723	Perm/Wash Buffer	100 mL	(none)
554714	BD Cytofix/Cytoperm™ Fixation/Permeablization Kit	250 Tests	(none)
554722	Fixation and Permeabilization Solution	125 mL	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 Tests	(none)
555028	BD Cytofix/Cytoperm Plus Kit (with BD GolgiPlug)	250 Tests	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Alaverdi N, Waters JB. *Pharmingen's Hotlines.* 1997:6-15. (Methodology)
BD Biosciences. Techniques for Immune Function Analysis, Application Handbook 1st Edition. 2003; Available: http://www.bdbiosciences.com/pdfs/manuals/02-8100055-21A1rr.pdf 2007, Jan. 25. (Methodology)
Lanier LL, Warner NL. Paraformaldehyde fixation of hematopoietic cells for quantitative flow cytometry (FACS) analysis. *J Immunol Methods.* 1981; 47(1):25-30. (Methodology)
Sander B, Andersson J, Andersson U. Assessment of cytokines by immunofluorescence and the paraformaldehyde-saponin procedure. *Immunol Rev.* 1991; 119:65-93. (Methodology)