

Technical Data Sheet

Fixation and Permeabilization Solution

Product Information

Material Number:	554722
Size:	125 mL
RRID:	AB_2869010

Description

BD Cytotfix/Cytoperm™ solution is supplied as a 1X solution and can be used for the simultaneous fixation and permeabilization of cells prior to intracellular cytokine staining.

Preparation and Storage

Store undiluted at 4°C.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
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Recommended Assay Procedure:

For the stimulation of cells, various *in vitro* methods have been reported for stimulating cells to produce cytokines. Polyclonal activators have been particularly useful for inducing cytokine-producing cells. These activators include the following: concanavalin A, lipopolysaccharide, phorbol esters plus calcium ionophore or ionomycin, phytohaemagglutinin, staphylococcus enterotoxin B, and monoclonal antibodies directed against subunits of the TCR/CD3 complex (with or without antibodies directed against costimulatory receptors, such as CD28).

1. Fix and Permeabilize Cells

- Thoroughly resuspend cells in 100 µL of BD Cytotfix/Cytoperm solution per well for microwell plates (or 250 µL for tubes) and incubate for 20 min. at 4°C. **Note:** Cell aggregation can be avoided by vortexing prior to the addition of the BD Cytotfix/Cytoperm™ solution.
- Wash cells two times in a buffer that contains a cell permeabilizing agent such as saponin (BD Perm/Wash™ buffer, Cat. 554723, which can be used as the wash buffer and as the antibody diluent).

2. Stain for Intracellular Cytokines

- Thoroughly resuspend fixed/permeabilized cells in 50 µL of a saponin-containing buffer (e.g., BD Perm/Wash™ buffer) containing a pre-determined optimal concentration of a fluorochrome-conjugated anti-cytokine antibody or appropriate negative control. Incubate at 4°C for 30 minutes in the dark. **Note:** Because saponin-mediated cell permeabilization is a reversible process, it is important to keep the cells in the presence of saponin during intracellular cytokine staining.
- Wash cells 2 times with saponin-containing buffer (e.g., BD Perm/Wash™ buffer) and resuspend in staining buffer prior to flow cytometric analysis.

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

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554722 Rev. 3



Danger: BD Cytotfix/Cytoperm™ Buffer (Fixation and Permeabilization Solution) contains 4.2% 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.
Harmful to aquatic life.

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.
Wash thoroughly after handling.
Do not breathe mist/vapours/spray.
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.
IF ON SKIN: Wash with plenty of water. Immediately call a POISON CENTER/doctor.
Wash contaminated clothing before reuse. Contaminated work clothing must not be allowed out of the workplace.
Avoid release to the environment.

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
554723	Perm/Wash Buffer	100 mL	(none)
554714	BD Cytotfix/Cytoperm™ Fixation/Permeablization Kit	250 Tests	(none)
554715	Fixation/Permeabilization Solution Kit with BD GolgiStop™	250 Tests	(none)
555028	Fixation/Permeabilization Solution Kit with BD GolgiPlug™	250 Tests	(none)

Product Notices

1. Please refer to wwwbdbiosciences.com/us/s/resources for technical protocols.

References

Assenmacher M, Schmitz J, Radbruch A. Flow cytometric determination of cytokines in activated murine T helper lymphocytes : expression of interleukin-10 in interferon-gamma and in interleukin-4-expressing cells. *Eur J Immunol.* 1994; 24(5):1097-1101. (Methodology)
Elson LH, Nutman TB, Metcalfe DD, Prussin C. Flow cytometric analysis for cytokine production identifies T helper 1, T helper 2, and T helper 0 cells within the human CD4+CD27- lymphocyte subpopulation. *J Immunol.* 1995; 154(9):4294-4301. (Methodology)
Jung T, Schauer U, Heusser C, Neumann C, Rieger C. Detection of intracellular cytokines by flow cytometry. *J Immunol Methods.* 1993; 159(1-2):197-207. (Methodology)
Prussin C, Metcalfe DD. Detection of intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. *J Immunol Methods.* 1995; 188(1):117-128. (Methodology)
Sander B, Andersson J, Andersson U. Assessment of cytokines by immunofluorescence and the paraformaldehyde-saponin procedure. *Immunol Rev.* 1991; 119:65-93. (Methodology)