Technical Data Sheet

Fixation and Permeabilization Solution

Product Information

Material Number: **554722**
Size: 125 mL

Description

BD Cytofix/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

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, phytohaemaglutinin, 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

a. Thoroughly resuspend cells in 100 µL of BD Cytofix/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 Cytofix/Cytoperm™ solution.
b. 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

a. 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.
b. 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 Cytofix/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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Danger

BD Cytofix/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.
May cause respiratory irritation.

Precautionary statements

Wear protective clothing / eye protection.
Wear protective gloves.
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.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554723	Perm/Wash Buffer	100 mL	(none)
554714	BD Cytofix/Cytoperm™ Fixation/Permeablization Kit	250 Tests	(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

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)

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