Technical Data Sheet

Fixation and Permeabilization Solution

Product Information

Material Number: **554722**

Size: 125 ml

Description

BD Cytofix/Cytoperm™ solution can be used for the simultaneous fixation and permeabilization of cells prior to intracellular cytokine staining.

Preparation and Storage

Store undiluted at 4° C.
Cytofix/Cytoperm solution is supplied as a 1X solution.

Application Notes

Recommended Assay Procedure:
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).

Procedure for Using Cytofix/Cytoperm: For specific methodology, please visit our web site, www.bdbiosciences.com, and go to the protocols section or the chapter on intracellular staining in the Immune Function Handbook.

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 1: 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 2: 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 (or BD Perm/Wash buffer) and resuspend in staining buffer prior to flow cytometric analysis.

NOTE 3: 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).

Warnings and Precautions: BD Cytofix/Cytoperm contains formaldehyde, a suspected carcinogen. Avoid contact with skin, eyes, and mucous membranes. Avoid breathing fumes. Harmful by inhalation, in contact with skin, and if swallowed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Use only in well-ventilated areas.

R40. Limited evidence of a carcinogenic effect.
R43. May cause sensitization by skin contact.
S2. Keep out of the reach of children.
S13 Keep away from food, drink and animal feedstuffs.
36/37 Wear suitable protective clothing and gloves.
46 If swallowed, seek medical advice immediately and show this container or label.
52 Not recommended for interior use on large surface areas.
56 Dispose of this material and its container at hazardous or special waste collection point.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
554723	Perm/Wash Buffer	250 tests	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. 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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