Technical Data Sheet

Perm/Wash Buffer

Product Information

Material Number: **554723**

Size: 100 ml

Description

The BD Perm/Wash™ buffer can be used in intracellular cytokine staining to permeabilize cells and to serve as an antibody diluent and cell wash buffer. 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.

Preparation and Storage

Store undiluted at 4° C.

Note: BD Perm/Wash buffer consists of 100 ml of concentrated stock solution (10X) containing both Fetal Bovine Serum (FBS) and saponin. It is not uncommon for the color of this product to vary from lot to lot. A small amount of insoluble precipitate is also common. Color variation and/or precipitate do not affect product performance. If desired, the precipitate can be removed before use by passing the diluted 1X BD Perm/Wash buffer through a 0.45 micron filter.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

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 BD Perm/Wash™ buffer:

1. Fix cells using a buffer containing paraformaldehyde, or use BD Cytofix/Cytoperm™ solution (Cat. No. 554722)
2. Permeabilize fixed cells by washing 2 times in 1X BD Perm/Wash buffer (Cat. No. 554723) (e.g., 1 ml/wash for staining in tubes and 250 µl/wash final volume for staining in microwell plates). Incubate for 15 minutes in 1X BD Perm/Wash buffer (the 15 minute incubation can be omitted if BD Cytofix/Cytoperm is used for fixing cells). Pellet cells.
   a. Dilute 10X BD Perm/Wash buffer in distilled H2O to make a 1X solution prior to use.
3. Stain for Intracellular Cytokines
   a. Thoroughly resuspend fixed/permeabilized cells in 50 µl of 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.
   b. Wash cells 2 times with 1X BD Perm/Wash buffer (1 ml/wash for staining in tubes and 250 µl/wash final volume for staining in microwell plates) and resuspend in staining buffer prior to flow cytometric analysis.

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

Warning: The BD Perm/Wash solution contains saponin and sodium azide which is known to be toxic. Avoid contact with skin, eyes and mucous membranes.

BD Biosciences

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Suggested Companion Products

Catalog Number	Name	Size	Clone
554722	Fixation and Permeabilization Solution	125 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. 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.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

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)

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Sander B, Andersson J, Andersson U. Assessment of cytokines by immunofluorescence and the paraformaldehyde-saponin procedure. *Immunol Rev.* 1991; 119:65-93.(Methodology)