## Technical Data Sheet

# **Fixation and Permeabilization Solution**

#### **Product Information**

 Material Number:
 554722

 Size:
 125 mL

#### Description

BD Cytofix/Cytoperm<sup>TM</sup> 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<sup>TM</sup> solution.
- b. Wash cells two times in a buffer that contains a cell permeabilizing agent such as saponin (BD Perm/Wash<sup>TM</sup> 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  $\mu$ L of a saponin-containing buffer (e.g., BD Perm/Wash<sup>TM</sup> buffer) containing a predetermined 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<sup>TM</sup> buffer) and resuspend in staining buffer prior to flow cytometric analysis.

*Note:* Both the BD Cytofix/Cytoperm<sup>™</sup> (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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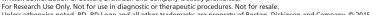
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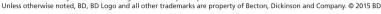
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#### Danger

BD Cytofix/Cytoperm<sup>TM</sup> 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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