

Technical Data Sheet

Protein Transport Inhibitor (Containing Brefeldin A)

Product Information

Material Number: 555029
Size: 1.0 ml

Description

The ex vivo addition of BD GolgiPlug™, a protein transport inhibitor containing brefeldin A, to in vitro- or in vivo-stimulated lymphoid cells blocks their intracellular protein transport processes. This results in the accumulation of cytokines and/or proteins in the Golgi complex. This increased accumulation of cytokines in the cell enhances the detectability of cytokine-producing cells with immunofluorescent staining and flow cytometric analysis.

Preparation and Storage

Store undiluted at 4°C.

Application Notes

Application

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

Stimulation of cells: Various *in vitro* methods have been reported for stimulated 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 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).

Procedure for using BD GolgiPlug™: Add 1 µl of BD GolgiPlug™ for every 1 ml of cell culture (e.g., $\sim 10^6$ cells/mL) and mix thoroughly. Treatment of stimulated cells for 4 to 6 hours with BD GolgiPlug™ significantly increases the ability to detect cytokine-producing cells by immunofluorescent staining. It is recommended that BD GolgiPlug™ not be kept in cell culture for longer than 12 hours.

As an alternative to BD GolgiPlug™, investigators may wish to consider using BD GolgiStop™, a protein transport inhibitor containing monensin (Cat. No. 554724). BD GolgiPlug™ and BD GolgiStop™ have been found to have differential effects on intracellular cytokine staining that is time, activator and cytokine dependent. These factors need to be considered when carrying out intracellular cytokine staining.

Warning: BD GolgiPlug™ contains brefeldin A, a chemical known to be harmful. Avoid contact with skin, eyes and mucous membranes. This product contains Dimethylsulfoxide, (DMSO). DMSO is considered an irritant.

R 36/37/38 Irritating to eyes, respiratory system and skin.

S23 Do not breathe gas/fumes/vapour/spray.

S24 Avoid contact with skin.

S25 Avoid contact with eyes.

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S28 After contact with skin, wash immediately with plenty of water.

S36/37 Wear suitable protective clothing and gloves.

S60 This material and its container must be disposed of as hazardous waste.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
555028	BD Cytofix/Cytoperm Plus Kit (with BD GolgiPlug)	250 tests	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharming/protocols for technical protocols.

References

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)

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