

Technical Data Sheet

Protein Transport Inhibitor (Containing Brefeldin A)

Product Information

Material Number: 555029
Size: 1 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

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| 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., ~10⁶ 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.

Suggested Companion Products

| Catalog Number | Name | Size | Clone |
|----------------|---|-----------|--------|
| 554724 | Protein Transport Inhibitor (Containing Monensin) | 0.7 mL | (none) |
| 555028 | BD Cytotfix/Cytoperm Plus Kit (with BD GolgiPlug) | 250 Tests | (none) |
| 554715 | BD Cytotfix/Cytoperm Plus Kit (with BD GolgiStop) | 250 Tests | (none) |

Product Notices

- Please refer to wwwbdbiosciences.com/pharming/en/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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