

Technical Data Sheet

Leukocyte Activation Cocktail, with BD GolgiPlug™

Product Information

Material Number: 550583

Size: 200 µL

Description

The most critical step for detection of intracellular accumulations of cytokines by intracellular staining is the isolation and activation of a cell population to induce production of the cytokine of interest. Without properly activated cytokine-producing cells, cultured in the presence of a protein transport inhibitor, the ability to detect intracellular accumulations of cytokines in most cases is severely compromised. 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, 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).

The Leukocyte Activation Cocktail, with BD GolgiPlug™ is a ready -to-use polyclonal cell activation mixture containing the phorbol ester, PMA (Phorbol 12-Myristate 13-Acetate), a calcium ionophore (Ionomycin) and the protein transport inhibitor BD GolgiPlug™ (Brefeldin A). This mixture may be utilized to elicit a primary cytokine response from T cells. Stimulation of cells using this cocktail results in cytokine production that is localized in the rough endoplasmic reticulum of cytokine -producing cells. This localization of cytokines is caused by the protein transport inhibitor component, Brefeldin A which blocks intracellular protein transport processes. The increased accumulation of cytokine protein levels in cells thus enhances detectability of cytokine-producing cells by immunofluorescent staining and flow cytometric analysis. The contents of each 100 µL vial is sufficient for treating up to 50 mL of cell cultures (at $\sim 10^6$ cells/mL); therefore one can stimulate $\geq 5 \times 10^7$ cells.

Preparation and Storage

Store product at -80°C prior to use or for long term storage of stock solutions.

Application Notes

Recommended Assay Procedure:

Procedure for Using Leukocyte Activation Cocktail, with BD GolgiPlug™ : Rapidly thaw the cocktail at 37°C in a water bath and add 2 µL of cocktail for every 1 mL of cell culture (e.g., $\sim 10^6$ cells/mL) and mix thoroughly. Place culture in a 37°C humidified CO2 incubator for 4-6 hr. Following activation, harvest and wash cells with FACS Staining Buffer (e.g., BD Pharmingen™ Stain Buffer, with FBS, Cat. No. 554656) for use in antibody staining protocols. Treatment of stimulated cells for 4 to 6 hours with Leukocyte Activation Cocktail, with BD GolgiPlug significantly increases the ability to detect cytokine-producing cells by immunofluorescent staining. It is recommended that Leukocyte Activation Cocktail, with BD GolgiPlug not be kept in cell culture for longer than 12 hours. BD GolgiPlug been found to have differential effects on intracellular cytokine staining that is time, activator and cytokine dependent, therefore the need to activate cells for periods longer than 4-6 hr may be determined empirically by the investigator.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to wwwbdbiosciences.com/pharming/en/protocols for technical protocols.

References

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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. (Biology)

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. (Biology)

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Vikingson A, Pederson K, Muller D. Enumeration of IFN-gamma producing lymphocytes by flow cytometry and correlation with quantitative measurement of IFN-gamma. *J Immunol Methods.* 1994; 173(2):219-228. (Biology)

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