Technical Data Sheet

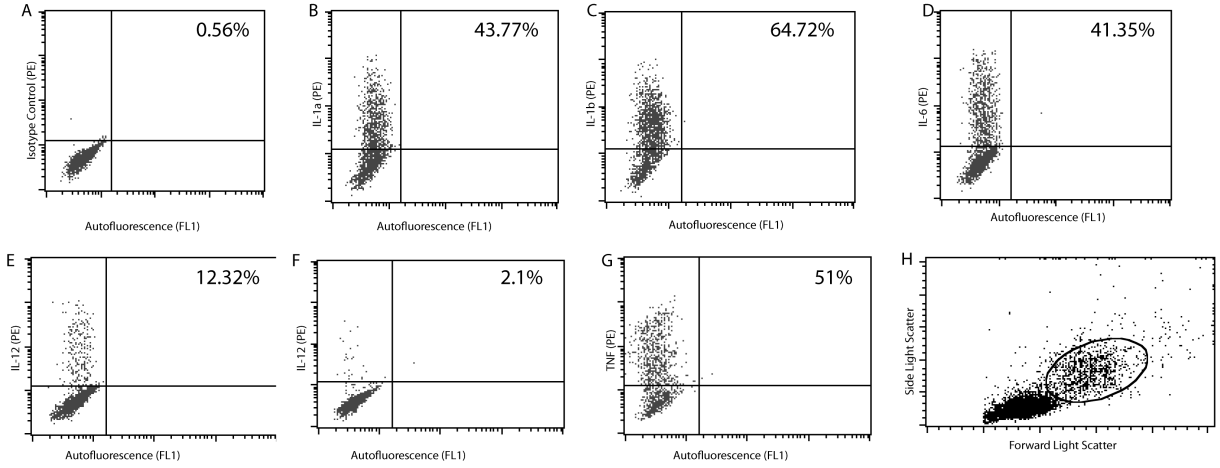
HiCK-3 Human Cytokine Positive Control Cells

Product Information

Material Number:	**555063**
Size:	1 mL
Concentration:	5x10^6 cells/ml
Storage Buffer:	Frozen in FBS and 10% DMSO.

Description

This suspension contains **H**uman **i**ntracellular **C**yto**K**ine-**3** (HiCK-3) Positive Control Cells. The HiCK-3 frozen cell suspension contains fixed, non-permeabilized human lymphoid cells. The suspension includes cells that express detectable levels of intracellular IL-1α, IL-1β, IL-6, IL-12 p40/p70, IL-12 p70, and TNF as determined by immunofluorescent intracellular cytokine staining and flow cytometry. HiCK-3 cell suspensions were prepared by stimulating human PBMCs in the presence of a protein transport inhibitor. After stimulation, the cells were harvested and fixed, then stored in 1 mL of 10% dimethylsulfoxide and 90% fetal bovine serum at -80°C. HiCK-3 cells contain a measurable proportion of cytokines, with representative flow cytometric data shown below. Performance from individual lots of HiCK-3 cells may differ due to donor variability. Investigators should anticipate similar, though not identical, results to those shown below.



Flow cytometric staining of HiCK-3 Human Cytokine Positive Cells for IL-1α, IL-1β, IL-6, IL-12 and TNF. *HiCK-3 cells were washed, permeabilized, and subsequently stained with either a PE-conjugated isotype control (upper far left panel), PE Mouse Anti-Human IL-1α (upper left middle panel, Cat. No. 554561), PE Mouse Anti-Human IL-1β (upper right middle panel, Cat. No. 340516), PE Rat Anti-Human IL-6 (upper far right panel, Cat. No. 554697), PE Mouse Anti-Human IL-12p40/p70 (lower far left panel, Cat. No. 554575), PE Rat Anti-Human IL-12 p70 (lower left middle panel, Cat. No. 557020), or PE Mouse Anti-Human TNF (lower right middle panel, Cat. No. 554513). Despite fixation and freezing, the side- and forward scattered light signals for these control cells (see far lower right panel) remain similar to those for freshly-prepared lymphoid cell preparations (data not shown). Quadrant markers were set based on the autofluorescence controls to calculate the percentages of cells contained in each quadrant region as shown.*

Preparation and Storage

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

Rapidly thaw and quick-spin product prior to use.

Avoid multiple freeze-thaws of product.

This preparation contains no preservatives, thus it should be handled under aseptic conditions.

After thawing and thoroughly resuspending cells with a pipette, “single-use” aliquots can be refrozen at -80°C and stored in polypropylene microtubes for use at a later time.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Flow Cytometry: HiCK-3 Cytokine Positive Control Cell suspensions contain intracellular IL-1α, IL-1β, IL-6, IL-12 p40/70, IL-12 p70, and TNF which have accumulated and are detectable by intracellular flow cytometric analysis. These cells may serve as a positive controls for verifying anti-cytokine antibody performance and/or the flow cytometric staining procedure itself (e.g. permeabilization). For flow cytometric staining, the frozen cell preparation should first be quickly and carefully thawed. Aliquots of the cell suspension can then be transferred to microwells or

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tubes. HiCK-3 cells are supplied fixed and non-permeabilized in dimethylsulfoxide (DMSO), so should be washed at least twice with staining buffer to remove the DMSO**. The cells must then be permeabilized by incubating for 10-15 min in BD Perm/Wash™ buffer (Cat. No. 554723), spun down to pellet and then followed by at least one wash in BD Perm/Wash™ buffer.** Cells can then be stained with either PE Mouse Anti-Human IL-1α (Cat. No. 554561), PE Mouse Anti-Human IL-1β (Cat. No. 340516), PE Rat Anti-Human IL-6 (Cat. No. 554697), PE Mouse Anti-Human IL-12 p40/p70 (Cat. No. 554575), PE Rat Anti-Human IL-12 p70 (Cat. No. 559325) or PE Mouse Anti-Human TNF (Cat. No. 554513).

Note: Cytokine-specific antibody staining of HiCK-3 cells can be demonstrated by preincubation of conjugated cytokine-specific antibody with recombinant cytokine or by pretreatment of the HiCK-3 cells with unlabeled (purified) blocking antibody. Investigators should note that variation with cell activation may contribute to suboptimal blocking.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554723	Perm/Wash Buffer	100 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)

Product Notices

1. This product contains human blood, serum, cells, or materials derived from them, which are potentially hazardous materials. Use universal precautions when handling. Handle as if product were capable of transmitting disease. Material used in this product has been tested using FDA approved methods and found negative for Human Immunodeficiency Virus (HIV-1/HIV-2), Hepatitis B Surface Antigen (HBSAG) and antibody to Hepatitis C Virus (HCV). However, no known test method can offer complete assurance that specimens of human origin will not transmit infectious disease. When handling or disposing, follow precautions described in CDC and FDA recommendations and OSHA Bloodborne Pathogen recommendations.
2. Avoid contact with skin and eyes.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

BD Biosciences. Techniques for Immune Function Analysis, Application Handbook 1st Edition. 2003; Available: http://www.bdbiosciences.com/pdfs/manuals/02-8100055-21A1rr.pdf 2007, Jan. 25. (Methodology: Flow cytometry)

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: IC/FCM Block)

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