

Technical Data Sheet

MiCK-3 Mouse Cytokine Positive Control Cells

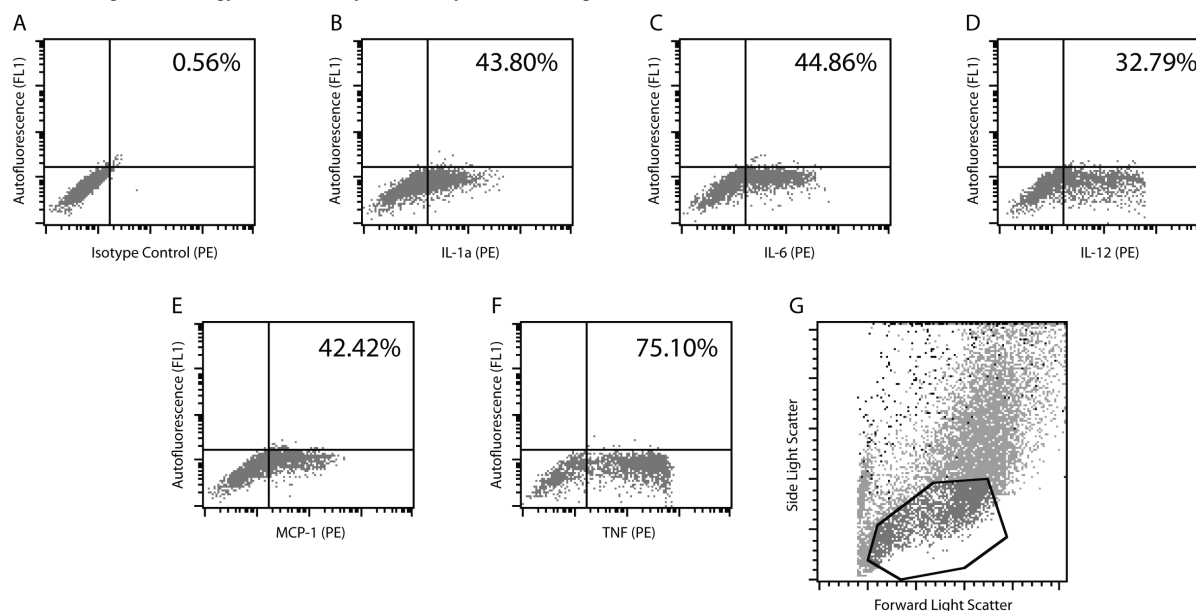
Product Information

Material Number:	554654
Size:	1 mL
Concentration:	5x10 ⁶ cells/ml
Storage Buffer:	Frozen in FBS and 10% DMSO.

Description

This suspension contains Mouse intracellular CytoKine-3 (MiCK-3) Positive Control Cells. The MiCK-3 cell suspension contains fixed, non-permeabilized mouse lymphoid cells. The suspension includes cells that express easily detectable levels of intracellular IL-1 α , IL-6, IL-12, MCP-1 or TNF as determined by immunofluorescent staining of intracellular cytokines and flow cytometry. MiCK-3 cell suspensions were prepared by stimulating mouse macrophages in the presence of a protein transport inhibitor. After stimulation, the cells were harvested and were incubated with Fc Block™ [rat IgG2b, κ anti-mouse CD16/CD32 (Fc γ II/III receptor) antibody; Cat. No. 553142] to reduce Fc receptor-mediated background staining. The cells were fixed and then stored in 10% dimethylsulfoxide (DMSO) and 90% fetal bovine serum at -80°C.

Each vial of MiCK-3 contains measurable proportions of cytokine-producing cells. Representative flow cytometric results are shown for typical staining of MiCK-3 Positive Control Cells. Investigators should anticipate similar (though not identical) results to those shown due to differences in staining methodology and in flow cytometers/cytometer settings.



Characteristic staining of MiCK-3 Positive Control Cells with anti-mouse IL-1 α , IL-6, IL-12, MCP-1 and TNF. MiCK-3 cells were washed, permeabilized, and subsequently stained with PE-rat IgG1 isotype control (PE-R3-34; Cat. No. 554685; see Panel A), PE-hamster anti-mouse IL-1 α antibody (PE-ALF-161, Cat. No. 559810, see Panel B), PE-rat anti-mouse IL-6 antibody (PE-MP5-20F3, Cat. No. 554401, see Panel C), PE-rat anti-mouse IL-12 p40/p70 (PE-C15.6, Cat. No. 554479; Panel D), PE-hamster anti-mouse MCP-1 (PE-2H5, Cat. No. 554443; Panel E) or PE-rat anti-mouse TNF (PE-MP6-XT22, Cat. No. 554419; Panel F). Despite fixation and freezing, the side- and forward-scattered light signals for these control cells (see Panel G) remain similar to those for freshly-prepared lymphoid cell preparations (data not shown). Quadrant markers were set based on the autofluorescence controls (and were verified using the recombinant cytokine blocking and unlabeled antibody blocking specificity controls, data not shown) to calculate the percentages of cells contained in each quadrant region as shown in Panels A-F).

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.

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Upon receipt, store the cell suspension at -80°C. Alternatively, the frozen cell suspension can be thawed and the capped vial should be quick spun to ensure complete retrieval of the contents from the vial. After thoroughly resuspending cells with a pipette, "single-use" aliquots can be refrozen and stored (-80°C) in polypropylene microtubes for a later time.

Application Notes

Application

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

MiCK-3 Positive Control Cell suspensions are intended to provide cells that contain intracellular accumulations of IL-1 α , IL-6, IL-12, MCP-1 or TNF which are easily detectable by immunofluorescent staining of intracellular cytokines and flow cytometry. As such, these cells serve as positive controls for verifying the binding activity of fluorescent anti-cytokine antibodies and the staining procedure itself (e.g., permeabilization). For staining, the frozen cell preparation should first be quickly and carefully thawed. Aliquots of the cell suspension can then be transferred to microwells or tubes. The fixed and non-permeabilized cells should be washed twice with staining buffer to remove the DMSO. The cells must then be incubated for 10 min followed by one wash in permeabilization/wash buffer (BD Perm/Wash™ Cat. No. 554723). The cells may then be stained with a fluorescent conjugate of either ALF-161 (hamster anti-mouse IL-1 α ; Cat. No. 559810), MP5-20F3 (rat anti-mouse IL-6; Cat. No. 554401), C15.6 (rat anti-mouse IL-12 p40/p70; Cat. No. 554479, or No. 554480), 2H5 (hamster anti-mouse MCP-1; Cat. No. 554443), MP6-XT22 (rat anti-mouse TNF; Cat. No. 554418, No. 554419 or No. 554420).

Note: Cytokine-specific staining on MiCK-3 cells can be demonstrated by preincubation of conjugated cytokine-specific antibody with recombinant cytokine or by pretreatment of the MiCK-3 cells with unlabeled blocking antibody. Due to the cell activation procedure, a small proportion of cells may stain non-specifically (i.e., not blocked with either unlabeled antibody or ligand).

Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
554723	Perm/Wash Buffer	100 mL	(none)
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2

Product Notices

1. Avoid contact with skin and eyes.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. 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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