Store at 4°C

Human T Cell Th1 Cytokine Response Flow Cytometry Panel

#28572

1 Kit
(50 assays)

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

Orders: 877-616-2355 (U.S.)
orders@cellsignal.com

For Research Use Only. Not For Use In Diagnostic Procedures.

Product Includes	Item #	Dilution	Species Reactivity
CD3 (UCHT1) Mouse mAb (PE Conjugate)	46233	1:20	H
CD4 (RPA-T4) Mouse mAb (violetFluor™ 450 Conjugate)	26755	1:20	H
CD8α (RPA-T8) Mouse mAb (PE-Cy7® Conjugate)	27442	1:20	H
TNF-α (D1G2) Rabbit mAb (Alexa Fluor® 647 Conjugate)	60959	1:50	H
IFN-γ (D3H2) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate)	12942	1:50	H

Description: The Human T Cell Th1 Cytokine Response Flow Cytometry Panel can be used to observe production of signature Th1 cytokines including IFN-γ and TNF-α.

T cells are identified by expression of CD3. There are two major subsets of conventional T cells: helper T cells which express CD4, and cytotoxic T cells which express CD8. Th1 cells are one of several subsets of helper T cell subsets. Th1 cells promote cell-mediated immunity to combat viral infection, intracellular bacteria, and tumor cells. Both Th1 and cytotoxic T cells can produce IFN-γ and TNF-α.

Specificity/Sensitivity: Each antibody in the Human T Cell Th1 Cytokine Response Flow Cytometry Panel detects endogenous levels of its target protein. CD3 (UCHT1) Mouse mAb (PE Conjugate) recognizes CD3ε. CD3 (UCHT1) Mouse mAb (PE Conjugate), CD4 (RPA-T4) Mouse mAb (violetFluor™ 450 Conjugate), and CD8α (RPA-T8) Mouse mAb (PE-Cy7® Conjugate) detect epitopes within the extracellular domains. TNF-α (D1G2) Rabbit mAb (Alexa Fluor® 647 Conjugate) and IFN-γ (D3H2) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate) detect epitopes within the intracellular domains.

Source/Purification: Monoclonal antibodies were purified from tissue culture supernatant via affinity chromatography. The purified antibodies were conjugated under optimal conditions, with unreacted dye removed from the preparation.

Directions for Use: Cytokine production by T cells can be induced by activating cells through a variety of methods. These include stimulation through the T cell receptor by incubation with an agonist CD3 antibody such as Human CD3ε Activating (OKT3) Mouse mAb (Low Endotoxin, Azide-free), or treatment with a combination of PMA (phorbol-12-myristate-13-acetate) and Ionomycin. The addition of Brefeldin A and Monensin during the cell stimulation will limit cytokine secretion and facilitate detection of intracellular cytokines. All antibodies in this kit are compatible with the Flow Cytometry Triton™ X-100 Permeabilization Protocol for Directly Conjugated Antibodies and can be used in a single staining mix on fixed and permeabilized cells. Prior to fixation and antibody incubation, we recommend adding a fixable viability dye such as the Ghost Dye™ Violet 510 Viability Dye to enable identification and exclusion of dead cells from analysis.

Gating strategy for observing production of Th1 cytokines by T cells: If a fixable viability dye was used, first gate on viable cells. Next, gate on lymphocytes based on forward scatter and side scatter. To observe cytokine production by helper T cells, gate on the CD3+CD4+ cells within the lymphocyte gate and then identify cells within this gate that are positive for staining of IFN-γ and/or TNF-α. To observe cytokine production by cytotoxic T cells, gate on the CD3+CD8+ cells within the lymphocyte gate and then identify cells within this gate that are positive for staining of IFN-γ and/or TNF-α.

Storage: CD3 (UCHT1) Mouse mAb (PE Conjugate), CD4 (RPA-T4) Mouse mAb (violetFluor™ 450 Conjugate), and CD8α (RPA-T8) Mouse mAb (PE-Cy7® Conjugate) are supplied in 10 mM NaH_2PO_4, 150 mM NaCl, 0.09% NaN_3, 0.1% gelatin, pH7.2. TNF-α (D1G2) Rabbit mAb (Alexa Fluor® 647 Conjugate) and IFN-γ (D3H2) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate) are supplied in PBS (pH 7.2), less than 0.1% sodium azide and 2 mg/ml BSA. Store at 4°C. *Do not aliquot the antibodies. Protect from light. Do not freeze.*

All components in this kit are stable in accordance with the date printed on the outer packaging label when stored at the recommended temperature. Please refer to product labels, datasheets, or web pages for specific "Best By" dates for each individual component.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Flow Cytometry Triton™ X-100 Permeabilization Protocol for Directly Conjugated Antibodies

A. Solutions and Reagents

All reagents required for this protocol may be efficiently purchased together in our Intracellular Flow Cytometry Kit (Triton™ X-100) #51995, or individually using the catalog numbers listed below.

Note: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **1X Phosphate Buffered Saline (PBS):** To prepare 1 L 1X PBS: add 100 ml 10X PBS (#12528) to 900 ml water, mix.
2. **4% Formaldehyde, Methanol-Free** (#47746)
3. **Cell Permeabilization Buffer:** Purchase ready-to-use (#39487) or to prepare 10 ml, add 30 µl Triton™ X-100 to 10 ml Antibody Dilution Buffer. Store at 4°C.
4. **Antibody Dilution Buffer:** Purchase ready-to-use Flow Cytometry Antibody Dilution Buffer (#13616), or to prepare 100 ml dissolve 0.5 g Bovine Serum Albumin (BSA) (#9998) in 100 ml 1X PBS. Store at 4°C.

Note: When including fluorescent cellular dyes in your experiment (including viability dyes, DNA dyes, etc.), please refer to the dye product page for the recommended protocol. Visit www.cellsignal.com for a full listing of cellular dyes validated for use in flow cytometry.

B. Fixation and Permeabilization

Note: Adherent cells or tissue should be dissociated and in single-cell suspension prior to fixation.

Note: Optimal centrifugation conditions will vary depending upon cell type and reagent volume. Generally, 150-300g for 1-5 minutes will be sufficient to pellet the cells.

Note: If using whole blood, lyse red blood cells and wash by centrifugation prior to fixation.

Note: Antibodies targeting CD markers or other extracellular proteins may be added prior to fixation if the epitope is disrupted by formaldehyde and/or Triton™ X-100. The antibodies will remain bound to the target of interest during the fixation and permeabilization process. Conduct a small-scale experiment if you are unsure.

1. Pellet cells by centrifugation and remove supernatant.
2. Resuspend cells in approximately 100 µl 4% formaldehyde per 1 million cells. Mix well to dissociate pellet and prevent cross-linking of individual cells.
3. Fix for 15 min at room temperature (20-25°C).
4. Wash by centrifugation with excess 1X PBS. Discard supernatant in appropriate waste container.
5. Resuspend cells in approximately 100 µl Cell Permeabilization Buffer per million cells.
6. Incubate for 10 minutes at room temperature.
7. Proceed with staining or store cells at 4°C in PBS overnight.

C. Immunostaining

Note: Count cells using a hemocytometer or alternative method.

1. Aliquot desired number of cells into tubes or wells. (Generally, $5x10^5$ to $1x10^6$ cells per assay).
2. Centrifuge cells and discard supernatant.
3. Resuspend cells in 100 µl of diluted antibody conjugates, prepared in Antibody Dilution Buffer at a recommended dilution or as determined via titration.
4. Incubate for 1 hr at room temperature (20-25°C). Protect from light.
5. Wash by centrifugation in Antibody Dilution Buffer or 1X PBS. Discard supernatant. Repeat.
6. Resuspend cells in 1X PBS and analyze on flow cytometer.