

Store at  
4°C

# Human Activated T Cell Markers Flow Cytometry Panel

#23428

1 Kit  
(100 assays)

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

**Orders:** 877-616-2355 (U.S.)  
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**For Research Use Only. Not For Use In Diagnostic Procedures.**

Product Includes	Item #	Dilution	Species Reactivity
CD3 (UCHT1) Mouse mAb (PE Conjugate)	46233	F, 1:20	H
CD4 (RPA-T4) Mouse mAb (violetFluor™ 450 Conjugate)	26755	F, 1:20	H
CD8α (RPA-T8) Mouse mAb (FITC Conjugate)	55397	F, 1:20	H
IL2-Rα/CD25 (BC96) Mouse mAb (APC Conjugate)	20164	F, 1:20	H
CD69 (FN50) Mouse mAb (PE-Cy7® Conjugate)	85313	F, 1:20	H

**Description:** The Human Activated T Cell Markers Flow Cytometry Panel can be used to identify activated human T cells.

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. Upon stimulation through the T cell receptor, T cells upregulate expression of CD69 and IL-2Rα/CD25. Regulatory T cells constitutively express IL2-Rα/CD25.

**Specificity/Sensitivity:** Each antibody in the Human Activated T Cell Markers Flow Cytometry Panel detects endogenous levels of its target protein. CD3 (UCHT1) Mouse mAb (PE Conjugate), CD4 (RPA-T4) Mouse mAb (violetFluor™ 450 Conjugate), CD8α (RPA-T8) Mouse mAb (FITC Conjugate), IL2-Rα/CD25 (BC96) Mouse mAb (APC Conjugate), and CD69 (FN50) Mouse mAb (PE-Cy7® Conjugate) detect epitopes within the extracellular 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.

**Storage:** Antibodies are supplied in 10 mM NaH<sub>2</sub>PO<sub>4</sub>, 150 mM NaCl, 0.09% NaN<sub>3</sub>, 0.1% gelatin, pH7.2. 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.

**Directions for Use:** All antibodies in this kit are compatible with the Flow Cytometry, Live Cell Protocol for Directly Conjugated Antibodies and can be used in a single staining mix. After antibody staining and prior to acquisition on a flow cytometer, we recommend adding Propidium Iodide or 7-AAD to enable identification and exclusion of dead cells from the analysis.

**Gating strategy for identifying activated T cells:**

If Propidium Iodide or 7-AAD was used, first gate on viable cells. Next, gate on lymphocytes based on forward scatter and side scatter. Helper T cells are the CD3+CD4+ cells within the lymphocyte gate. Activated helper T cells are positive for expression of CD69 and/or IL2-Rα/CD25. Regulatory T cells can also express IL2-Rα/CD25. Cytotoxic T cells are the CD3+CD8+ cells within the lymphocyte gate. Activated cytotoxic T cells are positive for expression of CD69 and/or IL2-Rα/CD25.

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## Flow Cytometry, Live Cell Protocol for Directly Conjugated Antibodies

### A. Solutions and Reagents

**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. Antibody Dilution Buffer:** Purchase ready-to-use Flow Cytometry Antibody Dilution Buffer (#13616), or prepare a 0.5% BSA PBS buffer by dissolving 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](http://www.cellsignal.com) for a full listing of cellular dyes validated for use in flow cytometry.

### B. Immunostaining

**NOTE:** Count cells using a hemocytometer or alternative method.

**NOTE:** If using whole blood, lyse red blood cells and wash by centrifugation prior to Immunostaining.

**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.

1. Aliquot desired number of cells into tubes or wells. (Generally,  $5 \times 10^5$  to  $1 \times 10^6$  cells per assay.)
2. Pellet cells by centrifugation and remove supernatant.
3. Resuspend cells in 100  $\mu$ l of diluted primary antibody, prepared in Antibody Dilution Buffer at a recommended dilution or as determined via titration.
4. Incubate for 30 min to 1 hr on ice. Protect from light.
5. Wash by centrifugation in Antibody Dilution Buffer. Discard supernatant. Repeat.
6. Resuspend cells in 200-500  $\mu$ l of Antibody Dilution Buffer and analyze on flow cytometer.