Store at 4°C

Mouse Naive/Effector/Memory T Cell Markers Flow Cytometry Panel

#78148

1 Kit
(200 assays)

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

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

New 06/21

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

Product Includes	Item #	Dilution	Species Reactivity
CD3 (17A2) Rat mAb (PE Conjugate)	28306	1:40	M
CD4 (RM4-5) Rat mAb (violetFluor™ 450 Conjugate)	92599	1:160	M
CD8α (2.43) Rat mAb (PE-Cy7® Conjugate)	87922	1:80	M
CD44 (IM7) Rat mAb (PE-Cy5® Conjugate)	94170	1:160	H, M
CD62L/L-Selectin (MEL-14) Rat mAb (FITC Conjugate)	76378	1:200	M
CD127/IL-7Rα (A7R34) Rat mAb (APC Conjugate)	28470	1:80	M

Description: The Mouse Naive/Effector/Memory T Cell Markers Flow Cytometry Panel can be used to distinguish naive, effector, and memory mouse T cells in both CD4 and CD8 T cell populations.

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. Naive, effector, effector memory, and central memory T cell states can be distinguished by differential expression patterns of CD44, CD62L, and CD127/IL-7Rα.

Specificity/Sensitivity: Each antibody in the Mouse Naive/Effector/Memory T Cell Markers Flow Cytometry Panel detects endogenous levels of its target protein and detects epitopes within the extracellular domain. CD3 (17A2) Rat mAb (PE Conjugate) recognizes endogenous levels of total CD3ε, CD3γ, and CD3δ proteins. CD44 (IM7) Rat mAb (PE-Cy5® Conjugate) is expected to detect all isoforms of CD44.

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_2PO_4, 150 mM NaCl, 0.09% NaN_3, 0.1% gelatin, pH 7.2. 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 a membrane impermeable viability dye such as Propidium Iodide or 7-AAD to enable identification and exclusion of dead cells from the analysis.

Gating strategy for observing naive, effector, and memory T cell subsets: 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. Cytotoxic T cells are the CD3+CD8+ cells within the lymphocyte gate. Next, observe expression of CD44 vs. CD62L on either helper T cells or cytotoxic T cells. Naive T cells are CD44-CD62L+. Central memory T cells are CD44+CD62L+. Effector memory and effector cells are CD44+CD62L-. To distinguish effector memory and effector cells, observe expression of CD127/IL-7Rα on the CD44+CD62L- population. Effector memory cells are positive for expression of CD127/IL-7Rα. Effector cells are negative for expression of CD127/IL-7Rα. Naive and central memory T cells also express CD127/IL-7Rα.

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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.

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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 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, $5x10^5$ to $1x10^6$ cells per assay.)
2. Pellet cells by centrifugation and remove supernatant.
3. Resuspend cells in 100 µl 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 µl of Antibody Dilution Buffer and analyze on flow cytometer.