Store at 4°C

Mouse Progenitor Exhausted CD8+ T Cell Markers Flow Cytometry Panel

#60395

1 Kit
(50 assays)

Cell Signaling TECHNOLOGY®

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

Product Includes	Item #	Dilution	Species Reactivity
PD-1 (Intracellular Domain) (D7D5W) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate)	34920	1:50	M
CD8α (2.43) Rat mAb (PE-Cy7® Conjugate)	87922	1:80	M
CD3 (17A2) Rat mAb (violetFluor™ 450 Conjugate)	38527	1:40	M
TCF1/TCF7 (C63D9) Rabbit mAb (PE Conjugate)	14456	1:50	H, M
Granzyme B (D2H2F) Rabbit mAb (Alexa Fluor® 647 Conjugate)	50590	1:50	H, M

Description: The Mouse Progenitor Exhausted CD8+ T Cell Markers Flow Cytometry Panel can be used to identify progenitor exhausted CD8+ T cells and their differentiated T cells with effector potential.

Cytotoxic T cells are identified by co-expression of CD3 and CD8α. Among these cells, the exhausted progenitor cells are TCF1+PD-1+ cells and are mostly Granzyme B-; the differentiated cells with effector potential are TCF1-PD-1+ and are mostly Granzyme B+. In response to immunotherapy, these progenitor TCF1+PD-1+CD8+ cells can expand and differentiate into TCF1-PD-1+CD8+ effector cells.

Specificity/Sensitivity: Each antibody in the Mouse Progenitor Exhausted CD8+ T Cell Markers Flow Cytometry Panel detects endogenous levels of its target protein. CD8α (2.43) Rat mAb (PE-Cy7® Conjugate) and CD3 (17A2) Rat mAb (violetFluor™ 450 Conjugate) detect epitopes within the extracellular domains. PD-1 (Intracellular Domain) (D7D5W) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate), TCF1/TCF7 (C63D9) Rabbit mAb (PE Conjugate), and Granzyme B (D2H2F) Rabbit mAb (Alexa Fluor® 647 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.

Storage: PD-1 (Intracellular Domain) (D7D5W) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate), TCF1/TCF7 (C63D9) Rabbit mAb (PE Conjugate), and Granzyme B (D2H2F) Rabbit mAb (Alexa Fluor® 647 Conjugate) are supplied in PBS (pH 7.2), less than 0.1% sodium azide and 2 mg/ml BSA. CD8α (2.43) Rat mAb (PE-Cy7® Conjugate) and CD3 (17A2) Rat mAb (violetFluor™ 450 Conjugate) 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 Intracellular Flow Cytometry Kit (Triton™ X-100) #51995 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.

This panel can be combined with peptide-MHC tetramer staining to identify antigen-specific cells. Tetramer staining is often performed on live cells. If tetramer staining is desired, we recommend comparing activity of the reagent in live cell staining vs. the recommended protocol for the panel. If there is not a significant difference between the two conditions, the tetramer can be included in the antibody panel staining mix. If there is significant loss of activity following fixation and permeabilization, tetramer staining should be done on live cells prior to fixation and permeabilization.

Gating strategy for observing progenitor exhausted CD8+ 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. Cytotoxic T cells are the CD3+CD8+ cells within the lymphocyte gate. Next, observe TCF1 and PD-1 expression on the cytotoxic T cells. Cytotoxic T cells that are TCF1+PD-1+ are exhausted progenitor cells and are expected to be mostly negative for Granzyme B expression. Cytotoxic T cells that are TCF1-PD-1+ are differentiated cells with effector potential and are expected to be mostly positive for expression of Granzyme B.

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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.