Store at 4°C

CAR-T Cell (G4S Linker) Transduction Efficiency Flow Cytometry Panel

#18839

1 Kit
(100 assays)

Cell Signaling TECHNOLOGY®

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Product Includes	Item #	Dilution	Species Reactivity
CD45 (HI30) Mouse mAb (redFluor 710 Conjugate)	39170	1:20	H
CD3 (UCHT1) Mouse mAb (PE-Cy7® Conjugate)	62670	1:20	H
CD4 (RPA-T4) Mouse mAb (FITC Conjugate)	48705	1:20	H
CD8α (RPA-T8) Mouse mAb (PE Conjugate)	88829	1:20	H
CD14 (61D3) Mouse mAb (violetFluor 450 Conjugate)	82944	1:20	H
G4S Linker (E7O2V) Rabbit mAb (Alexa Fluor® 647 Conjugate)	69782	1:50	All

Description: The CAR-T Cell (G4S Linker) Transduction Efficiency Flow Cytometry Panel can be used to identify conventional human T cell subsets that have been engineered to express scFv-based CARs containing a G4S linker.

CD45 is a pan leukocyte marker. 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. Monocytes are identified by the expression of CD14. CAR positive cells are identified by the expression of a G4S linker.

Specificity/Sensitivity: Each of the lineage marker antibodies in the CAR-T Cell (G4S Linker) Transduction Efficiency Flow Cytometry Panel detects endogenous levels of its target protein. CD3 (UCHT1) Mouse mAb (PE-Cy7® Conjugate) detects an epitope within the extracellular domain of CD3ε protein. CD4 (RPA-T4) Mouse mAb (FITC Conjugate), CD8α (RPA-T8) Mouse mAb (PE Conjugate), CD45 (HI30) Mouse mAb (redFluor 710 Conjugate), and CD14 (61D3) Mouse mAb (violetFluor 450 Conjugate) detect epitopes within the extracellular domains. G4S Linker (E7O2V) Rabbit mAb (Alexa Fluor® 647 Conjugate) recognizes exogenously expressed levels of scFv-based CARs containing a G4S linker.

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.

Gating strategy for identifying viable, CAR positive T cell subsets: Gate on live cells using Propidium Iodide or 7-AAD as a histogram or scatterplot with forward scatter (FSC). Use a gate set on side scatter (SSC) vs. FSC to exclude cell debris. Singlets may be identified by setting a gate on FSC-H vs. FSC-A. Next, use a plot of CD45 vs. CD3 to identify CD45+ leukocytes, and CD3+ and CD3- subpopulations. Observe G4S Linker vs. CD3 expression and set a gate on CD3+G4S Linker+ cells, which are CAR+ T cells. Observe CD4 vs. CD8 expression within the gated population to identify CD4+ and CD8+ T cells that are CAR+. Similarly, observe G4S Linker vs. CD14 expression within the CD3- gate to identify contaminating monocytes.

Storage: CD3 (UCHT1) Mouse mAb (PE-Cy7® Conjugate), CD4 (RPA-T4) Mouse mAb (FITC Conjugate), CD8α (RPA-T8) Mouse mAb (PE Conjugate), CD45 (HI30) Mouse mAb (redFluor 710 Conjugate), and CD14 (61D3) Mouse 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. G4S Linker (E7O2V) Rabbit mAb (Alexa Fluor® 647 Conjugate) is 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.

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.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **FC-FP**— Flow cytometry-Fixed/Permeabilized **FC-L**— Flow cytometry-Live **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, 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 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, 150-300 g for 1-5 min 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.