Store at 4°C

Human Natural Killer Cell Markers Flow Cytometry Panel

#83586

1 Kit
(100 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
CD45 (HI30) Mouse mAb (FITC Conjugate)	86532	1:20	H
CD3 (UCHT1) Mouse mAb (violetFluor™ 450 Conjugate)	61347	1:20	H
NCAM1 (CD56) (MY31) Mouse mAb (APC Conjugate)	51997	1:20	H
CD16 (3G8) Mouse mAb (PE Conjugate)	82004	1:20	H

Description: The Human Natural Killer Cell Markers Flow Cytometry Panel can be used to detect human natural killer (NK) cells.

Human natural killer cells are CD45+CD3-CD56+. The large subset with high CD16 expression are mature cytotoxic natural killer cells, while those with low CD16 expression are immature precursors and cytokine producers.

Specificity/Sensitivity: Each antibody in the Human Natural Killer Cell Markers Flow Cytometry Panel detects endogenous levels of its target protein and 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_2PO_4, 150 mM NaCl, 0.09% NaN_3, 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 human natural killer cells: If Propidium Iodide or 7-AAD was used, first gate on viable cells. Next, gate based on CD45 expression, then gate on lymphocytes based on forward scatter and side scatter on CD45+ fraction. NK cells are CD3-CD56+, and these cells can further be analyzed for CD16 expression.

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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, 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.