Store at 4°C

Human Monocyte STING Activation Flow Cytometry Panel

#69210

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
CD45 (HI30) Mouse mAb (violetFluor 450 Conjugate)	74292	1:20	H
CD11b/ITGAM (M1/70) Rat mAb (FITC Conjugate)	24442	1:100	H, M
HLA-DR (L243) Mouse mAb (PerCP Conjugate)	17634	1:20	H
CD14 (61D3) Mouse mAb (redFluor 710 Conjugate)	64342	1:100	H
CD16 (3G8) Mouse mAb (PE Conjugate)	82004	1:20	H
Phospho-STING (Ser366) (D8K6H) Rabbit mAb (Alexa Fluor® 647 Conjugate)	43499	1:50	H

Description: The Human Monocyte STING Activation Flow Cytometry Panel includes antibodies targeting phospho-STING (Ser366) and phenotyping markers to enable observation of STING activation in human monocyte populations among peripheral blood mononuclear cells (PBMCs).

CD45 is a pan leukocyte marker. Monocytes and dendritic cells (DCs) are identified by co-expression of CD11b and HLA-DR. Monocytes can be distinguished from DCs by expression of CD14. Classical monocytes are CD14+CD16-, intermediate monocytes are CD14+CD16+, and nonclassical monocytes are CD14dimCD16+. DCs are CD14-CD16-. STING mediates the innate immune response to DNA. STING is activated upon binding of the second messenger cGAMP, which is produced by cGAS. Binding of cGAMP to cGAS triggers conformational changes in STING, trafficking of STING from the ER to the Golgi, and recruitment of TBK1, which phosphorylates STING at Ser366.

Specificity/Sensitivity: Each antibody in the Human Monocyte STING Activation Flow Cytometry Panel detects endogenous levels of its target protein. CD45 (HI30) Mouse mAb (violetFluor 450 Conjugate), CD11b/ITGAM (M1/70) Rat mAb (FITC Conjugate), HLA-DR (L243) Mouse mAb (PerCP Conjugate), CD14 (61D3) Mouse mAb (redFluor 710 Conjugate), and CD16 (3G8) Mouse mAb (PE Conjugate) detect epitopes within the extracellular domain. Phospho-STING (Ser366) (D8K6H) Rabbit mAb (Alexa Fluor® 647 Conjugate) recognizes endogenous levels of STING protein only when phosphorylated at Ser366.

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 observing STING phosphorylation in monocyte populations: If a fixable viability dye was used, first gate on viable cells. Next, gate on CD45+ immune cells. Viewing the CD45+ population, gate on singlets using appropriate scatter parameters, such as FSC-A vs. FSC-H. Observe CD11b vs. HLA-DR expression and gate on CD11b+HLA-DR+ monocytes and DCs. Observe CD14 vs. CD16 on the gated population. Classical monocytes are CD14+CD16-, intermediate monocytes are CD14+CD16+, nonclassical monocytes are CD14dimCD16+, and DCs are CD14-CD16-. Monocytic MDSCs are CD11b+HLA-DR-CD14+ and can also be observed. Observe phospho-STING (Ser366) in populations of interest.

Storage: CD45 (HI30) Mouse mAb (violetFluor 450 Conjugate), CD11b/ITGAM (M1/70) Rat mAb (FITC Conjugate), HLA-DR (L243) Mouse mAb (PerCP Conjugate), CD14 (61D3) Mouse mAb (redFluor 710 Conjugate), and CD16 (3G8) Mouse mAb (PE Conjugate) are supplied in 10 mM NaH_2PO_4, 150 mM NaCl, 0.09% NaN_3, 0.1% gelatin, pH 7.2. Phospho-STING (Ser366) (D8K6H) 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 4ºC. *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: STING activation can be induced through treatment with a variety of STING agonists including Poly(dA:dT) Sodium Salt #47945 and the second messenger 2',3'-cGAMP (sodium salt) #35573. 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 Fixable Viability Dye #59863 to enable identification and exclusion of dead cells from 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.

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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.