

Store at
4°C

Human B Cell Signaling Flow Cytometry Panel

#31328

1 Kit
(50 assays)

Support: +1-978-867-2388 (U.S.)
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
CD19 (Intracellular Domain) (D4V4B) XP [®] Rabbit mAb (Alexa Fluor [®] 647 Conjugate)	16344	1:50	H, M
Phospho-CD79A (Tyr182) (D1B9) Rabbit mAb (Alexa Fluor [®] 488 Conjugate)	52821	1:50	H
Phospho-Syk (Tyr525/526) (C87C1) Rabbit mAb (PE Conjugate)	6485	1:50	H
CD45 (HI30) Mouse mAb (violetFluor 450 Conjugate)	74292	1:20	H

Description: The Human B Cell Signaling Flow Cytometry Panel can be used to interrogate activation of human B cells among peripheral blood mononuclear cells (PBMCs).

CD45 is a pan leukocyte marker. B cells are identified by expression of CD19. Engagement of the B cell receptor (BCR) triggers the tyrosine phosphorylation of CD79A at Tyr182, which is mediated by Src family kinases. Following engagement of the BCR, Syk is recruited to phosphorylated ITAM motifs within the BCR signaling complex, which facilitates Syk activation through phosphorylation of Tyr525/526 within the kinase activation loop.

Specificity/Sensitivity: Each antibody in the Human B Cell Signaling Flow Cytometry Panel detects endogenous levels of its target protein. CD45 (HI30) Mouse mAb (violetFluor 450 Conjugate) detects an epitope within the extracellular domain. CD19 (Intracellular Domain) (D4V4B) XP[®] Rabbit mAb (Alexa Fluor[®] 647 Conjugate) detects an epitope within the intracellular domain. Phospho-CD79A (Tyr182) (D1B9) Rabbit mAb (Alexa Fluor[®] 488 Conjugate) recognizes endogenous levels of human CD79A protein only when phosphorylated at Tyr188. This corresponds to Tyr182 of mouse CD79A protein. Phospho-Syk (Tyr525/526) (C87C1) Rabbit mAb (PE Conjugate) detects endogenous levels of Syk protein only when phosphorylated at Tyr525/526 of human Syk. It also detects Syk protein when singly phosphorylated at Tyr526 of human Syk. It does not cross-react with other tyrosine-phosphorylated protein tyrosine kinases.

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 B cell receptor activation in B cell populations:

Apply the same gating strategy for both untreated and treated cell populations. If a fixable viability dye was used, then 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 CD19 vs. SSC-A and gate on CD19+ B cells. Observe phospho-CD79A (Tyr182) and phospho-Syk (Tyr525/526) within the CD19+ gate.

Storage: CD45 (HI30) Mouse mAb (violetFluor 450 Conjugate) is supplied in 10 mM NaH₂PO₄, 150 mM NaCl, 0.09% NaN₃, 0.1% gelatin, pH 7.2. CD19 (Intracellular Domain) (D4V4B) XP[®] Rabbit mAb (Alexa Fluor[®] 647 Conjugate), Phospho-CD79A (Tyr182) (D1B9) Rabbit mAb (Alexa Fluor[®] 488 Conjugate), and Phospho-Syk (Tyr525/526) (C87C1) Rabbit mAb (PE Conjugate) are 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: B cell receptor activation can be induced through treatment with Goat Anti-Human IgM, F(ab')₂ Antibody (Low Endotoxin, Azide-free) #96232. All antibodies in this kit are compatible with the Intracellular Flow Cytometry Kit (Methanol) #13593 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.

U.S. Patent No. 7,429,487, foreign equivalents, and child patents deriving therefrom.

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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, Methanol 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 (Methanol) #13593**, or individually using the catalog numbers listed below.

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

- 1X Phosphate Buffered Saline (PBS):** To prepare 1 L 1X PBS: add 100 mL 10X PBS (#12528) to 900 mL water mix.
- 4% Formaldehyde, Methanol-Free (#47746)**
- 100% Methanol (#13604):** Chill before use.
- 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. Fixation

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, 150-300 g for 1-5 min 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 methanol. The antibodies will remain bound to the target of interest during the fixation and permeabilization process. However, note that some fluorophores (including PE and APC) are damaged by methanol and thus should not be added prior to permeabilization. Conduct a small-scale experiment if you are unsure.

- Pellet cells by centrifugation and remove supernatant.
- Resuspend cells in approximately 100 μ L 4% formaldehyde per 1 million cells. Mix well to dissociate pellet and prevent cross-linking of individual cells.
- Fix for 15 min at room temperature (20-25°C).
- Wash by centrifugation with excess 1X PBS. Discard supernatant in appropriate waste container. Resuspend cells in 0.5-1 mL 1X PBS. Proceed to Permeabilization step.

a. Alternatively, cells may be stored overnight at 4°C in 1X PBS.

C. Permeabilization

- Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol.
- Permeabilize for a minimum of 10 min on ice.
- Proceed with immunostaining (Section D) or store cells at -20°C in 90% methanol.

D. Immunostaining

NOTE: Count cells using a hemocytometer or alternative method.

- Aliquot desired number of cells into tubes or wells. (Generally, 5×10^5 to 1×10^6 cells per assay.)
- Wash cells by centrifugation in excess 1X PBS to remove methanol. Discard supernatant in appropriate waste container. Repeat if necessary.
- Resuspend cells in 100 μ L of diluted primary antibody, prepared in Antibody Dilution Buffer at a recommended dilution or as determined via titration.
- Incubate for 1 hr at room temperature. Protect from light.
- Wash by centrifugation in Antibody Dilution Buffer or 1X PBS. Discard supernatant. Repeat.
- Resuspend cells in 200-500 μ L of 1X PBS and analyze on flow cytometer.