Store at 4°C

Protein L (APC Conjugate)

#29480

Cell Signaling TECHNOLOGY®

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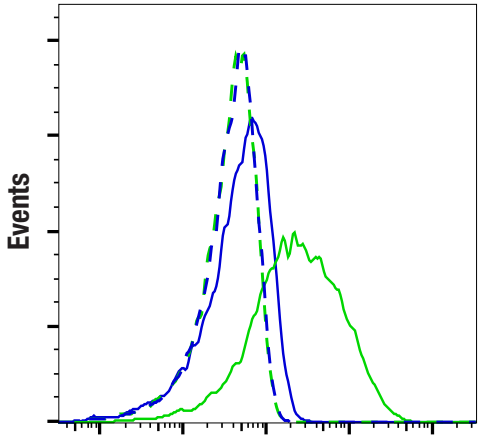
Description: This Cell Signaling Technology® product is conjugated to allophycocyanin (APC) under optimal conditions.

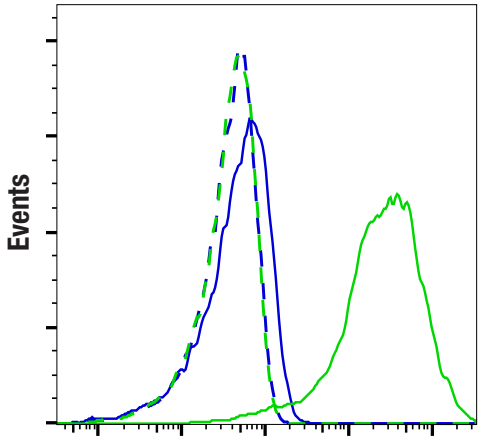
Background: Protein L was first isolated from the surface of bacterial species *Peptostreptococcus magnus* (1). Unlike Protein A and Protein G which differentially bind to the Fc region of immunoglobulin isotype heavy chains, Protein L contains Ig binding domains that interact with the κ light chain variable domain without disrupting paratope function (2-4). This attribute not only facilitates binding to a broader range of immunoglobulin classes and subclasses than either Protein A or Protein G but also allows for binding to Fab fragments and single chain variable fragments (scFvs) that contain κ light chain sequences. Protein L does, however, display specificity with respect to κ light chain subtypes. In humans, Protein L binds to VκI, VκIII, and VκIV subtypes, whereas binding is restricted to VκI in mice (4).

Specificity/Sensitivity: Conjugation to APC makes this product ideally suited for direct flow cytometric analysis of cells expressing κ light chain-containing immunoglobulins as well as immunoglobulin fragments that contain κ light chains, such as scFvs.

Source/Purification: Protein L (APC Conjugate) is recombinantly derived and genetically engineered to remove the cell wall-, cell membrane-, and albumin-binding regions of Protein L, enhancing binding to immunoglobulins. The purified Protein L was conjugated under optimal conditions, with unreacted dye removed from the preparation.



Flow cytometric analysis of live Jurkat cells (blue) or live Jurkat cells engineered to stably express an scFv-based Anti-CD19 CAR (green) using Protein L (APC Conjugate) (solid lines) or unstained Jurkat cells (dashed lines). CAR cell line was provided by the Lohmueller Lab, University of Pittsburgh.



Flow cytometric analysis of live Jurkat cells (blue) or live Jurkat cells engineered to stably express an scFv-based Anti-CD20 CAR (green) using Protein L (APC Conjugate) (solid lines) or unstained Jurkat cells (dashed lines). CAR cell line was provided by the Lohmueller Lab, University of Pittsburgh.

Storage: Supplied in PBS (pH 7.2), less than 0.1% sodium azide, and 2 mg/mL BSA. Store at 4°C. *Do not aliquot. Protect from light. Do not freeze.*

Directions for Use: Recommended Dilution: 1:50

Please visit cellsignal.com for validation data and a complete listing of recommended companion products.

Background References:

(1) Liebermann, B. et al. (1990) *Dtsch Z Mund Kiefer Gesichtschir* 14, 418-23.

(2) Nilson, B.H. et al. (1992) *J Biol Chem* 267, 2234-9.

(3) Akerström, B. and Björck, L. (1989) *J Biol Chem* 264, 19740-6.

(4) Nilson, B.H. et al. (1993) *J Immunol Methods* 164, 33-40.

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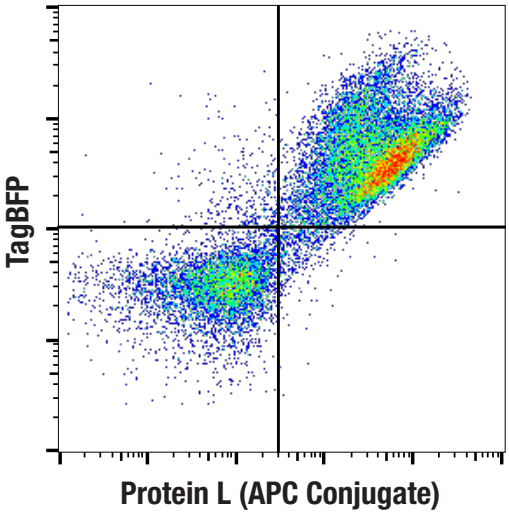
Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **FC-FP**— Flow cytometry-Fixed/Permeabilized **FC-L**— Flow cytometry-Live **E-P**—ELISA-Peptide
Species Cross-Reactivity: **H**—human **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 cytometric analysis of a mixed population containing live wild-type Jurkat cells and live Jurkat cells engineered to stably express an scFv-based Anti-CD20 CAR, using Protein L (APC Conjugate). Tag Blue fluorescent protein (TagBFP) is co-expressed with the CAR. CAR cell line was provided by the Lohmueller Lab, University of Pittsburgh.

Flow Cytometry, Live Cell Protocol for Directly Conjugated Protein L

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. **Dilution Buffer:** Purchase ready-to-use Flow Cytometry Antibody Dilution Buffer (#13616) that is compatible with Protein L, 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 Protein L conjugate, prepared in 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 Dilution Buffer. Discard supernatant. Repeat. If using a fluorescent conjugate of Protein L, skip to step 9.
6. For labeling biotinylated Protein L, resuspend cells in 100 µL of diluted fluorochrome-conjugated streptavidin or anti-biotin antibody (prepared in Dilution Buffer at the recommended dilution).
7. Incubate for 30 min on ice. Protect from light.
8. Wash by centrifugation in Dilution Buffer. Discard supernatant. Repeat.
9. Resuspend cells in 200-500 µL of Dilution Buffer and analyze on a flow cytometer.

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