Technical Data Sheet

PE Mouse anti-SLP-76 (pY128)

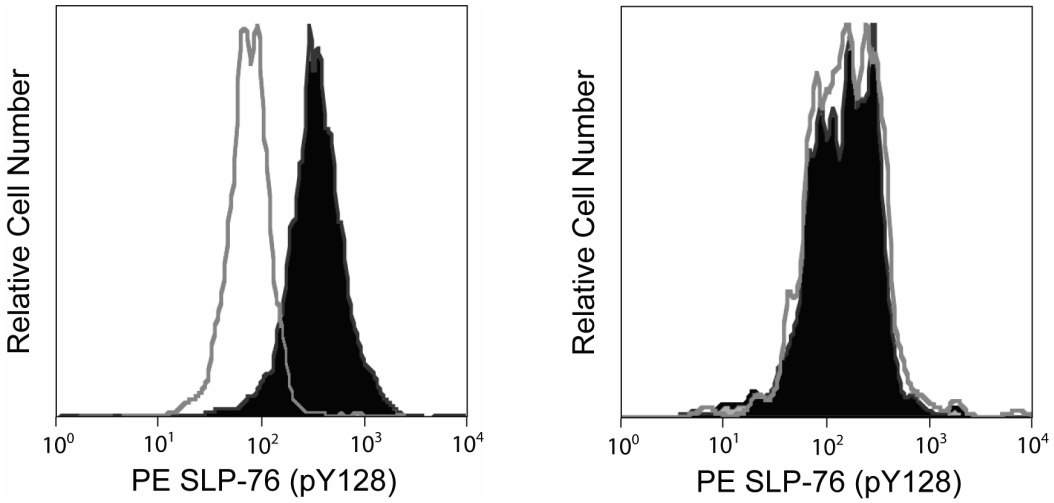
Product Information

Material Number:	**558437**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	J141-668.36.58
Immunogen:	Phosphorylated Human SLP-76
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	Confirmed: Human, Mouse Predicted: Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

SLP-76 (*S*H2 domain-containing *L*eukocyte *P*rotein of *76* kDa) is a tyrosine phosphoprotein that is involved in the T cell receptor (TCR) -mediated intracellular signaling pathway. It may be involved in the signaling pathways of other peripheral blood leukocytes; thymic/splenic cells; and in human T, B, and monocytic cell lines. SLP-76 consists of several motifs that signify its importance in protein-protein interactions involved in intracellular signaling pathways, such as the SH2 domain in the C-terminus, the three amino-terminus 17-amino acid repeats with conserved tyrosine and acidic residues (DYE(S/P)P), and a proline rich region. SLP-76 has been shown to associate with Gads, Grb2, PLCγ1, SLAP-130, and Vav, all of which are part of the signaling cascade in T lymphocytes. An early event in the T cell activation pathway is the phosphorylation, by the Syk-family kinase ZAP-70, of SLP-76 at the three conserved tyrosine motifs, which then mediate interactions with downstream effectors. The phosphorylated tyrosine 128 (Y128) brings the Rho family guanine nucleotide exchange factor Vav1 and the Nck adapter protein, which binds to p21-activated kinase (PAK1) and Wiskott-Aldrich syndrome protein (WASP), into the activation complex. Vav1, PAK1, and WASP may mediate TCR-stimulated actin cytoskeletal rearrangement.

The J141-688.36.58 monoclonal antibody recognizes the phosphorylated Y128 of activated SLP-76.



Analysis of SLP-76 (pY128) in human peripheral blood lymphocytes. *Human peripheral blood mononuclear cells were either stimulated by cross-linking of CD3 and CD28 with NA/LE Mouse anti-Human CD3 mAb UCHT1 (Cat. No. 555329) and NA/LE Mouse anti-Human CD28 mAb CD28.2 (Cat. No. 555725) on ice for 15 minutes followed by Purified Goat anti-Mouse Ig (Cat. No. 553998) on ice for 15 minutes, and then allowed to undergo phosphorylation at 37°C for 5 minutes (shaded histograms) or unstimulated (open histograms). The cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes at 37°C, permeabilized (BD™ Phosflow Perm Buffer III, Cat. No. 558050) on ice for 30 minutes, blocked with normal mouse immunoglobulin, and then stained with PE Mouse anti-SLP-76 (pY128) and PerCP-Cy5.5 Mouse anti-human CD3 mAb SK7 (Cat. No. 340949). The left panel displays the upregulated phosphorylation of SLP-76 in activated CD3-positive T lymphocytes, while the right panel displays the lack of upregulated SLP-76 phosphorylation in CD3-negative non-T cells. Flow cytometry was performed on a BD™ FACSCalibur flow cytometry system.*

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Preparation and Storage

The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Tested

Recommended Assay Procedure:

This antibody conjugate is suitable for intracellular staining of human whole blood and mouse splenocytes (using BD™ Phosflow Lyse/Fix Buffer) and peripheral blood mononuclear cells (using BD Cytofix™ Fixation Buffer). Any of the three BD™ Phosflow permeabilization buffers may be used.

Suggested Companion Products

Catalog Number	Name	Size	Clone
558049	Lyse/Fix Buffer 5X	250 ml	(none)
558050	Perm Buffer III	125 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
558052	Perm Buffer II	125 ml	(none)
554655	Fixation Buffer	100 ml	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

Fang N, Motto DG, Ross SE, Koretzky GA. Tyrosines 113, 128, and 145 of SLP-76 are required for optimal augmentation of NFAT promoter activity. *J Immunol.* 1996; 157:3769-3773. (Biology)

Janssen E, Zhang W. Adaptor proteins in lymphocyte activation. *Curr Opin Immunol.* 2003; 15:269-276. (Biology)

Wu JN, Koretzky GA. The SLP-76 family of adapter proteins. *Semin Immunol.* 2004; 16:379-393. (Biology)