Technical Data Sheet

Alexa Fluor® 647 Mouse anti-SHP2 (pY542)

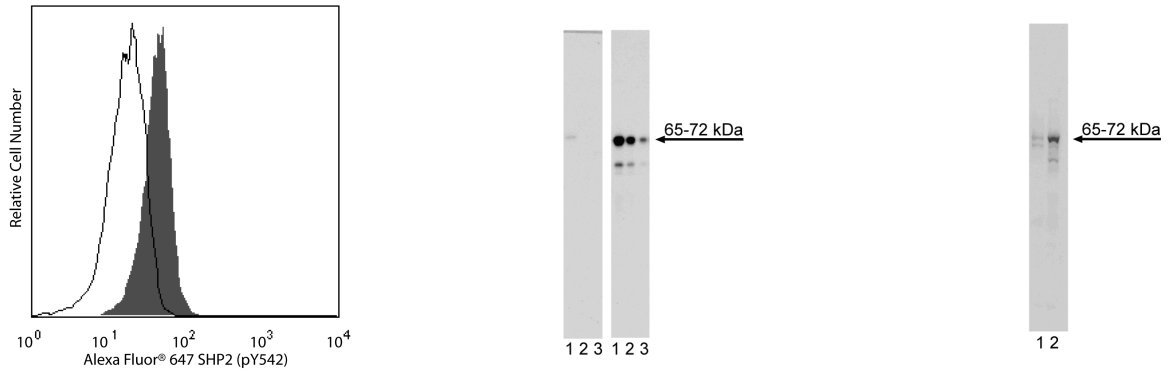
Product Information

Material Number:	**560390**
Alternate Name:	PTN11, PTP1D, PTP2C, PTPN11, SH-PTP2, SH-PTP3, SHP-2, SHPTP2
Size:	50 Tests
Vol. per Test:	20 µl
Clone:	L99-921
Immunogen:	Phosphorylated Human SHP2 Peptide
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Mouse Predicted Reactivity: Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

SHP2 is a member of the cytosolic class of protein-tyrosine phosphatases (PTPs). SHP2 reportedly contains two SH2 domains, both of which are N-terminal to the PTP catalytic domain. SH2 PTPs are believed to work in conjunction with protein-tyrosine kinases to maintain intracellular protein phosphotyrosine homeostasis and cell cycle progression. The expression of SHP2 has been reported to be highest in brain, heart, and kidney. SHP2 is phosphorylated at two C-terminal tyrosine residues: Y542 and Y580. Phosphorylation of Y542 creates an interaction in the N-terminal SH2 domain and appears to relieve basal inhibition of the PTP, while phosphorylation at Y580 creates an interaction at the C-terminal that stimulates PTP activity.

The L99-921 monoclonal antibody recognizes the phosphorylated Y542 of SHP2 (PTP2C isoform 2). The homologous phosphorylation site in the PTP2Ci splice variant (isoform 1) is Y546. The specificity of this antibody was validated by confirming, using western blot analysis, that RNA-mediated interference (RNAi) of the specific protein was able to down-regulate the expression of SHP2 (pY542).



Analysis of SHP2 (pY542) in activated human lymphocytes. *Left panel: Peripheral blood mononuclear cells (PBMC) 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 1-2 minutes (shaded histogram) or unstimulated (open histogram). The PBMC were then fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655) for 10 minutes at 37ºC, permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050) on ice for at least 30 minutes, blocked with normal mouse immunoglobulin, and then stained with Alexa Fluor® 647 Mouse anti-SHP2 (pY542). For data analysis, lymphocytes were selected by their scatter profile. Flow cytometry was performed on a BD FACSCanto™ II flow cytometry system.*

The specificity of mAb L99-921 was confirmed by western blot analysis using unconjugated antibody on lysates from mouse and human cells. *Middle panel: Lysates from mouse NIH-3T3 cells that had been treated with 200 nM PDGF (Cat. No. 354051) for 30 minutes (right blot) or untreated (left blot) were probed with purified mouse anti-SHP2 (pY542) monoclonal antibody at concentrations of 1.0, 0.25 and 0.063 µg/ml (Lanes 1, 2, and 3, respectively). SHP2 (pY542) is identified as a band of 65-72 kDa in the treated cells. Right panel: Lysates from PBMCs that were untreated (lane 1) or stimulated by cross-linking of CD3 and CD28 (lane 2) were probed with 0.5 µg/ml purified mouse anti-SHP2 (pY542) monoclonal antibody. SHP2 (pY542) is identified as a band of 65-72 kDa, with increased intensity in the stimulated cells.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

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The purified or conjugated mAb was characterized by flow cytometry (Flow) and western blot (WB) using these model systems:

Method	Species	Cells	Treatment	Fixation	Perm buffer	Result
Flow	Human	PBMC	CD3/CD28 crosslinking	Cytofix	Perm I, II, or III	Up-regulated expression
	Mouse	NIH/3T3	PDGF	Cytofix	Perm III	Up-regulated expression
WB	Human	HeLa S3	none or un-related RNAi			65-72-kDa band observed
			SHP2 RNAi			65-72-kDa band reduced
		PBMC	none			65-72-kDa band observed
			CD3/CD28 crosslinking			65-72-kDa band increased
	Mouse	NIH/3T3	PDGF			65-72-kDa band induced

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
Western blot	Tested During Development

Recommended Assay Procedure:

This antibody conjugate is suitable for intracellular staining of cell lines 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
554655	Fixation Buffer	100 mL	(none)
557885	Perm/Wash Buffer I	125 mL	(none)
558052	Perm Buffer II	125 mL	(none)
558050	Perm Buffer III	125 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
555329	Purified NA/LE Mouse Anti-Human CD3	0.5 mg	UCHT1
555725	Purified NA/LE Mouse Anti-Human CD28	0.5 mg	CD28.2
553998	Polyclonal Goat Anti-Mouse Ig	0.5 mg	Polyclonal

Product Notices

1. 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).
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
4. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
5. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
6. Ficoll-Paque is a trademark of Amersham Biosciences Limited.
7. 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.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
9. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
10. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.

References

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Bennett AM, Tang TL, Sugimoto S, Walsh CT, Neel BG. Protein-tyrosine-phosphatase SHPTP2 couples platelet-derived growth factor receptor beta to Ras. *Proc Natl Acad Sci U S A.* 1994; 91(15):7335-7339. (Biology)

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Marie-Cardine A, Kirchgessner H, Bruyns E et al. SHP2-interacting transmembrane adaptor protein (SIT), a novel disulfide-linked dimer regulating human T cell activation. *J Exp Med.* 1999; 189(8):1181-1194. (Biology)

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