Technical Data Sheet

PE Mouse anti-Zap70 (pY292)

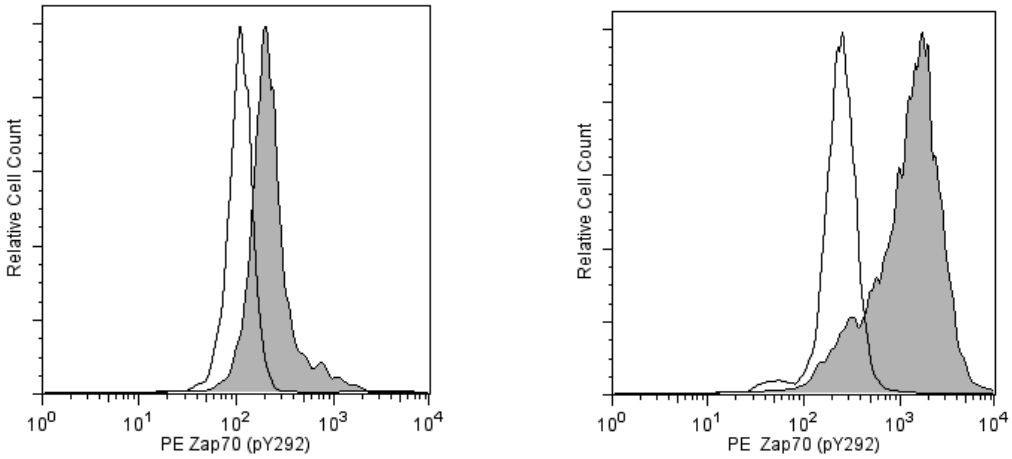
Product Information

Material Number:	**558510**
Alternate Name:	ZAP-70 (pY292)
Size:	50 tests
Vol. per Test:	20 µl
Clone:	J34-602
Immunogen:	Phosphorylated Human ZAP70
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The 70-kDa ζ chain-associated protein (ZAP70) is a Syk-family protein tyrosine kinase (PTK) that associates with the ζ subunit of the T cell antigen receptor (TCR) and undergoes tyrosine phosphorylation following TCR stimulation. ZAP70 contains two SH2-like domains with the PTK domain located at the C-terminus. TCR-mediated Lck activity leads to phosphorylation of ZAP70 on Tyrosine 493 in the regulatory loop of the PTK domain leading to upregulation of ZAP70 kinase activity. Tyrosine 292 (Y292), in the linker region between the SH2 and PTK domains, is autophosphorylated by the activated PTK domain. By binding with c-Cbl, the phosphorylated Y292 can down-regulate TCR signaling.

The J34-602 antibody recognizes ZAP70 phosphorylated at Y292.



LEFT: Analysis of ZAP70 (pY292) in activated human T lymphocytes. *Human whole blood was either stimulated by cross-linking of CD3 with NA/LE Mouse anti-Human CD3 mAb UCHT1 (Cat. No. 555329) 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 histogram), or unstimulated (open histogram). The cells were lysed and fixed with 1X BD Phosflow™ Lyse/Fix Buffer (Cat. No. 558049) for 10-15 minutes at 37ºC, 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-ZAP70 (pY292) and PerCP-Cy5.5 Mouse anti-human CD3 mAb SK7 (Cat. No. 340949). Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system. For data analysis, CD3-positive and -negative lymphocytes were selected by their scatter and staining profiles. The figure displays the CD3-positive T lymphocytes. Up-regulated phosphorylation of ZAP70 (pY292) was not observed in the CD3-negative lymphocytes (data not shown).*

RIGHT: Analysis of ZAP70 (pY292) in activated human T leukemia cells. *Jurkat cells (ATCC TIB152) 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 histogram), or unstimulated (open histogram). 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 at least 30 minutes, blocked with normal mouse immunoglobulin, and then stained with PE Mouse anti-ZAP70 (pY292). Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

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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 with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

This antibody conjugate is suitable for intracellular staining of human whole blood (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
554655	Fixation Buffer	100 ml	(none)
558049	Lyse/Fix Buffer 5X	250 ml	(none)
558050	Perm Buffer III	125 ml	(none)
558052	Perm Buffer II	125 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)

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. 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.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.

References

Kong G, Dalton M, Wardenburg JB, Straus D, Kurosaki T, Chan AC. Distinct tyrosine phosphorylation sites in ZAP-70 mediate activation and negative regulation of antigen receptor function. *Mol Cell Biol.* 1996; 16:5026-5035. (Biology)

Magnan A, Di Bartolo V, Mura AM, et al. T cell development and T cell responses in mice with mutations affecting tyrosines 292 or 315 of the ZAP-70 protein tyrosine kinase. *J Exp Med.* 2001; 194:491-505. (Biology)

Rao N, Lupher ML Jr, Ota S, Reedquist KA, Druker BJ, Band H. The linker phosphorylation site Tyr292 mediates the negative regulatory effect of Cbl on ZAP-70 in T cells. *J Immunol.* 2000; 164:4616-4626. (Biology)

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