Technical Data Sheet

PE Mouse Anti-Zap70 (Y319)/Syk (Y352)

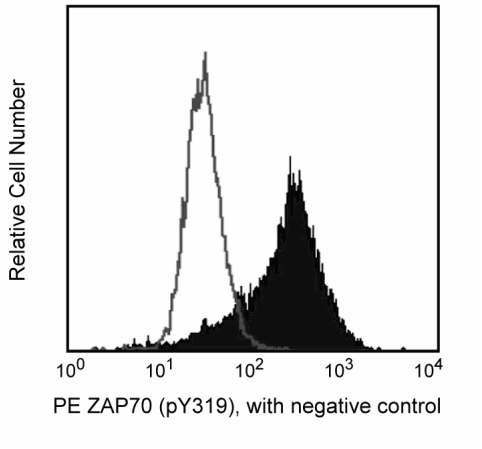
Product Information

Material Number:	**557881**
Alternate Name:	ZAP-70; SRK; STD; TZK; Zeta-chain associated protein kinase, 70kD
Size:	50 Tests
Vol. per Test:	20 µl
Clone:	17A/P-ZAP70
Immunogen:	Human phosphorylated ZAP70 Peptide
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Tested in Development: Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

ZAP70 is a protein tyrosine kinase (PTK) that associates with the z 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. It appears that both ZAP70 and Syk are recruited to the phosphorylated CD3 and z subunits after TCR stimulation. TCR stimulation leads to autophosphorylation of ZAP70 at Tyr-315 amd Tyr-319, and mutation of the Tyr-319 site dramatically impairs TCR signaling. In addition, TCR-mediated Lck activity leads to phosphorylation of ZAP70 on Tyr-493 in the regulatory loop of the kinase domain leading to upregulation of ZAP70 kinase activity. The significance of ZAP70 activation in mediating TCR signal transduction has been confirmed by showing that ZAP70 activity is absent in an autosomal recessive form of severe combined immunodeficiency (SCID). This is due to mutations affecting the ZAP70 kinase domain which affect the stability of the protein and TCR signaling.

Clone 17A/P-ZAP70 recognizes the phosphorylated form of ZAP70 (Y319). It also cross-reacts with SYK (Y352) due to homology of the phosphorylation site with ZAP70 (Y319). The PE-conjugated format has been evaluated using human and mouse model systems. The unconjugated form of the antibody (Cat. No. 612574) has also been shown to work in western blot analysis on human, mouse, and rat cells.



Flow cytometric analysis of Phospho-ZAP70. *Jurkat cells (ATCC TIB 152) were starved overnight in RPMI containing 0.1% FCS. The following day, cells were either left untreated (unshaded) or treated (shaded) with H2O2 (5 mM for 15 minutes at 37°C). Cells were fixed in 2% paraformaldehyde (10 minutes at 37°C) and permeabilized by adding 90% methanol to the cell pellet (30 minutes on ice or overnight at -20°C). Cells were then washed twice in BD Pharmingen™ Stain Buffer (Cat. No. 554656), and stained with PE Mouse Anti-Zap70 (Y319)/Syk (Y352). The cells were analyzed on a BD FACSCalibur™ flow cytometry system.*

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:

Jurkat cells treated with H2O2 are suggested as a positive control. However, other cell types or methods may also be used for detection of phosphorylated ZAP70.

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.268.5430	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**

Investigators are encouraged to reference http://www.bdbiosciences.com/research/ics/resources/index.jsp for more information concerning BD Phosflow™ protocols and other resources.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
554655	Fixation Buffer	100 mL	(none)
558050	Perm Buffer III	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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
4. 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.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. 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

Arpaia E, Shahar M, Dadi H, Cohen A, Roifman CM. Defective T cell receptor signaling and CD8+ thymic selection in humans lacking zap-70 kinase. *Cell.* 1994; 76(5):947-958. (Biology)

Chan AC, Kadlecek TA, Elder ME, et al. ZAP-70 deficiency in an autosomal recessive form of severe combined immunodeficiency. *Science.* 1994; 264:1599-1601. (Biology)

Di Bartolo V, Mege D, Germain V, et al. Tyrosine 319, a newly identified phosphorylation site of ZAP-70, plays a critical role in T cell antigen receptor signaling. *J Biol Chem.* 1999; 274(10):6285-6294. (Biology)

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.268.5430	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**