

Technical Data Sheet

Purified Mouse Anti-Human ZAP-70 (pY319)/Syk (pY352)**Product Information**

Material Number:	612575
Size:	150 µg
Concentration:	250 µg/ml
Clone:	17A/P-ZAP70
Immunogen:	Human phosphorylated ZAP70 Peptide
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Tested in Development: Mouse, Rat
Target MW:	70 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

ZAP70 is a 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. It appears that both ZAP70 and Syk are recruited to the phosphorylated CD3 and ζ subunits after TCR stimulation. TCR stimulation leads to autophosphorylation of ZAP70 at Tyr-315 and 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.



Jurkat cells were treated with 1 mM pervanadate for 15 minutes at 37°C, then either left untreated (lane 1) or treated (lane 2) with 50 µg/ml of alkaline phosphatase for 30 minutes at 37°C. The top panel was probed with ZAP70 (Cat. No. 610239) and the bottom was probed with ZAP70 (pY319) (Cat. No. 612574).

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. Store undiluted at -20° C.

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Application Notes

Application

Western blot	Routinely Tested
Flow cytometry	Tested During Development

Recommended Assay Procedure:

For flow cytometry we recommend to use conjugated versions of ZAP70 (pY319)/Syk (pY352) antibody: cat. No. 557817 (Alexa Fluor® 647 ZAP70 (pY319)/Syk (pY352)), 557818 (Alexa Fluor® 488 ZAP70 (pY319)/Syk (pY352)), 557881 (PE ZAP70 (pY319)/Syk (pY352)). For purified version AP70 (pY319)/Syk (pY352) as a second step for flow cytometry, we recommend to use of clone A85-1 in either FITC (cat. No. 553443) or PE (cat. No. 550083).

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharming/en/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.

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, Kadlecěk 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)