

p-ZAP-70 (pY319.17A): sc-136248

BACKGROUND

The activation of T lymphocytes by antigens is mediated by the T cell receptor (TCR), which is a multisubunit complex assembled from at least six different genes. The TCR subunits include the T α and T β chains, the CD3 γ , δ and ϵ chains and a ζ -containing homodimer or heterodimer. The protein tyrosine kinase ZAP-70 binds to the phosphorylated immunoreceptor tyrosine-base activation motifs (ITAMs) of the TCR ζ chain through two Src-homology (SH2) domains. This binding results in the phosphorylation of ZAP-70 on multiple tyrosine residues, including Tyr 292 and Tyr 319. ZAP-70 is autophosphorylated on Tyr 292, which is thought to negatively regulate ZAP-70 function in lymphocytes. Alternatively, ZAP-70 is positively regulated by phosphorylation on Tyr 319, which mediates the SH2-dependent interaction between Lck and ZAP-70.

REFERENCES

- Clevers, H., et al. 1988. The T cell receptor/CD3 complex: a dynamic protein ensemble. *Annu. Rev. Immunol.* 6: 629-662.
- Frank, S.J., et al. 1990. The structure and signaling function of the invariant T cell receptor components. *Semin. Immunol.* 2: 89-97.
- Watts, J.D., et al. 1994. Identification by electrospray ionization mass spectrometry of the site of tyrosine phosphorylation induced in activated Jurkat T cells on the protein tyrosine kinase ZAP-70. *J. Biol. Chem.* 269: 29520-29529.
- Zhao, Q. and Weiss, A. 1996. Enhancement of lymphocyte responsiveness by a gain-of-function mutation of ZAP-70. *Mol. Cell. Biol.* 16: 6765-6774.
- Magistrelli, G., et al. 1999. Role of the Src homology 2 domains and interdomain regions in ZAP-70 phosphorylation and enzymatic activity. *Eur. J. Biochem.* 266: 1166-1173.
- Di Bartolo, V., et al. 1999. Tyrosine 319, a newly identified phosphorylation site of ZAP-70, plays a critical role in T cell antigen receptor signaling. *J. Biol. Chem.* 274: 6285-6294.
- Pelosi, M., et al. 1999. Tyrosine 319 in the interdomain B of ZAP-70 is a binding site for the Src homology 2 domain of Lck. *J. Biol. Chem.* 274: 14229-14237.

CHROMOSOMAL LOCATION

Genetic locus: ZAP70 (human) mapping to 2q11.2, SYK (human) mapping to 9q22.2; Zap70 (mouse) mapping to 1 B, Syk (mouse) mapping to 13 A5.

SOURCE

p-ZAP-70 (pY319.17A) is a mouse monoclonal antibody raised against a short amino acid sequence containing Tyr 319 phosphorylated ZAP-70 of human origin.

PRODUCT

Each vial contains 200 μ g IgG $_1$ kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-136248 P, (100 μ g peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% stabilizer protein).

APPLICATIONS

p-ZAP-70 (pY319.17A) is recommended for detection of Tyr 319 phosphorylated ZAP-70 and Tyr 352 phosphorylated Syk of mouse, rat and human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) and immunoprecipitation [1-2 μ g per 100-500 μ g of total protein (1 ml of cell lysate)].

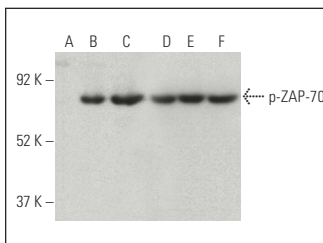
Molecular Weight of p-ZAP-70: 70 kDa.

Positive Controls: Jurkat whole cell lysate: sc-2204 or Jurkat + pervanadate cell lysate: sc-24716.

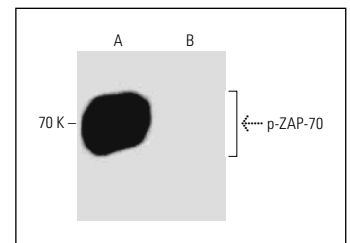
RECOMMENDED SUPPORT REAGENTS

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent), Lambda Phosphatase: sc-200312A and Western Blotting Luminol Reagent: sc-2048. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml).

DATA



p-ZAP-70 (pY319.17A): sc-136248. Western blot analysis of ZAP-70 phosphorylation in untreated (A, D), hydrogen peroxide treated (B, E) and pervanadate treated (C, F) Jurkat whole cell lysates. Antibodies tested include p-ZAP-70 (pY319.17A): sc-136248 (A, B, C) and ZAP-70 (1E7.2): sc-32760 (D, E, F). Blocked with UltraCruz® Blocking Reagent: sc-516214. Detection reagent used: m-IgG κ BP-HRP: sc-516102.



p-ZAP-70 (pY319.17A): sc-136248. Western blot analysis of ZAP-70 phosphorylation in Jurkat whole cell lysates treated with 1 mM pervanadate for 15 minutes at 37°C, then either left untreated (A) or treated with 50 μ g/ml of alkaline phosphatase for 30 minutes at 37°C (B).

SELECT PRODUCT CITATIONS

- Jang, I.K., et al. 2010. GRB2 functions at the top of the T-cell antigen receptor-induced tyrosine kinase cascade to control thymic selection. *Proc. Natl. Acad. Sci. USA* 107: 10620-10625.
- Yang, F., et al. 2022. A novel TLR4-SYK interaction axis plays an essential role in the innate immunity response in bovine mammary epithelial cells. *Biomedicines* 11: 97.

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

RESEARCH USE

For research use only, not for use in diagnostic procedures.