

p-ZAP-70 (Tyr 319)-R: sc-12946-R

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 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

1. Clevers, H., et al. 1988. The T cell receptor/CD3 complex: a dynamic protein ensemble. *Annu. Rev. Immunol.* 6: 629-662.
2. Frank, S.J., et al. 1990. The structure and signaling function of the invariant T cell receptor components. *Semin. Immunol.* 2: 89-97.
3. 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.
4. Zhao, Q., et al. 1996. Enhancement of lymphocyte responsiveness by a gain-of-function mutation of ZAP-70. *Mol. Cell Biol.* 16: 6765-6774.
5. Magistrelli, G., et al. 1999. Role of the Src homology 2 domains and inter-domain regions in ZAP-70 phosphorylation and enzymatic activity. *Eur. J. Biochem.* 266: 1166-1173.

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 (Tyr 319)-R is a rabbit polyclonal antibody raised against a short amino acid sequence containing phosphorylated Tyr 319 of ZAP-70 of human origin.

PRODUCT

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

Blocking peptide available for competition studies, sc-12946 P, (100 μ g peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% BSA).

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.

APPLICATIONS

p-ZAP-70 (Tyr 319)-R 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), immunoprecipitation [1–2 μ g per 100–500 μ g of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

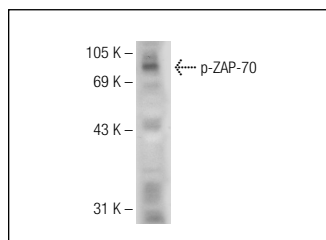
Molecular Weight of p-ZAP-70: 70 kDa.

Positive Controls: Jurkat whole cell lysate: sc-2204.

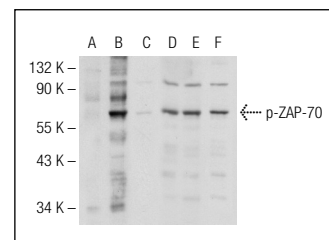
RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent), Western Blotting Luminol Reagent: sc-2048 and Lambda Phosphatase: sc-200312A. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

DATA



p-ZAP-70 (Tyr 319)-R: sc-12946-R. Western blot analysis of ZAP-70 phosphorylation in Jurkat whole cell lysate.



Western blot analysis of ZAP-70 phosphorylation in untreated (A,D), pervanadate treated (B,E) and pervanadate and lambda protein phosphatase (sc-200312A) treated (C,F) Jurkat whole cell lysates. Antibodies tested include p-ZAP-70 (Tyr 319)-R: sc-12946-R (A,B,C) and ZAP-70 (LR): sc-574 (D,E,F).

SELECT PRODUCT CITATIONS

1. Sela, U., et al. 2006. The negative regulators Foxj1 and Foxo3a are upregulated by a peptide that inhibits systemic lupus erythematosus-associated T cell responses. *Eur. J. Immunol.* 36: 2971-2980.
2. Lee, H.S., et al. 2008. CEACAM1 dynamics during *Neisseria gonorrhoeae* suppression of CD4⁺ T lymphocyte activation. *J. Immunol.* 180: 6827-6835.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.