

p-ZAP-70 (Tyr 493): sc-33526

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

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.

CHROMOSOMAL LOCATION

Genetic locus: ZAP70 (human) mapping to 2q11.2; Zap70 (mouse) mapping to 1 B.

SOURCE

p-ZAP-70 (Tyr 493) is a rabbit polyclonal antibody raised against a short amino acid sequence containing Tyr 493 phosphorylated 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-515648 P, (100 μ g peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% stabilizer protein).

APPLICATIONS

p-ZAP-70 (Tyr 493) is recommended for detection of Tyr 493 phosphorylated ZAP-70 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).

Suitable for use as control antibody for ZAP-70 siRNA (h): sc-29526, ZAP-70 siRNA (m): sc-36867, ZAP-70 shRNA Plasmid (h): sc-29526-SH, ZAP-70 shRNA Plasmid (m): sc-36867-SH, ZAP-70 shRNA (h) Lentiviral Particles: sc-29526-V and ZAP-70 shRNA (m) Lentiviral Particles: sc-36867-V.

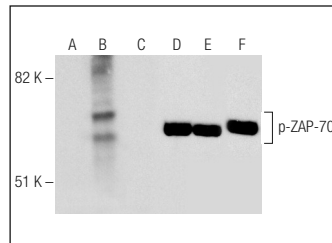
Molecular Weight of p-ZAP-70: 70 kDa.

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

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



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 493): sc-33526 (A, B, C) and ZAP-70 (1E7.2): sc-32760 (D, E, F).

SELECT PRODUCT CITATIONS

1. Su, Y., et al. 2011. Receptor desensitization and blockade of the suppressive effects of prostaglandin E2 and adenosine on the cytotoxic activity of human melanoma-infiltrating T lymphocytes. *Cancer Immunol. Immunother.* 60: 111-122.
2. Lo Buono, N., et al. 2011. The CD157-integrin partnership controls transendothelial migration and adhesion of human monocytes. *J. Biol. Chem.* 286: 18681-18691.
3. Govers, C., et al. 2014. TCRs genetically linked to CD28 and CD3 ϵ do not mispair with endogenous TCR chains and mediate enhanced T cell persistence and anti-melanoma activity. *J. Immunol.* 193: 5315-5326.

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.

PROTOCOLS

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