

ZAP-70 siRNA (m): sc-36867

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 Ti α and β chains, the CD3 γ , δ and ϵ chains and a ζ -containing homodimer or heterodimer. The disulfide-linked Ti α - β heterodimer is responsible for antigen recognition, but the short 5 amino acid cytoplasmic domains of Ti α and β are unlikely to be sufficient to couple to intracellular signaling pathways. In contrast, the structured features of the CD3 and ζ subunits suggest a role in signal transduction. Of these, the ζ chain, which is expressed as either a homodimer or heterodimer, has a short extracellular domain of only 9 amino acids, but a larger 113 amino acid cytoplasmic domain. A tyrosine phosphoprotein, ZAP-70, has been identified that associates with ζ and undergoes tyrosine phosphorylation following TCR stimulation.

REFERENCES

1. Clevers, H., et al. 1988. The T cell receptor/CD3 complex: a dynamic protein ensemble. *Annu. Rev. Immunol.* 6: 629-662.
2. Baniyash, M., et al. 1988. Disulfide linkage of the ζ and η chains of the T cell receptor. *J. Biol. Chem.* 263: 9874-9878.
3. Baniyash, M., et al. 1988. The T cell antigen receptor ζ chain is tyrosine phosphorylated upon activation. *J. Biol. Chem.* 263: 18225-18230.
4. Baniyash, M., et al. 1989. The isolation and characterization of the murine T cell antigen receptor ζ chain gene. *J. Biol. Chem.* 264: 13252-13257.
5. Frank, S.J., et al. 1990. The structure and signaling function of the invariant T cell receptor components. *Semin. Immunol.* 2: 89-97.
6. Clayton, L.K., et al. 1991. CD3 η and CD3 ζ are alternatively spliced products of a common genetic locus and are transcriptionally and/or post-transcriptionally regulated during T-cell development. *Proc. Natl. Acad. Sci. USA* 88: 5202-5206.
7. Chan, A.C., et al. 1991. The TCR ζ chain associates with a tyrosine kinase upon TCR stimulation associates with ZAP-70, a 70K M_r tyrosine phosphoprotein. *Proc. Natl. Acad. Sci. USA* 88: 9166-9170.

CHROMOSOMAL LOCATION

Genetic locus: Zap70 (mouse) mapping to 1 B.

PRODUCT

ZAP-70 siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ZAP-70 shRNA Plasmid (m): sc-36867-SH and ZAP-70 shRNA (m) Lentiviral Particles: sc-36867-V as alternate gene silencing products.

For independent verification of ZAP-70 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-36867A, sc-36867B and sc-36867C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20°C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20°C , avoid contact with RNases and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

ZAP-70 siRNA (m) is recommended for the inhibition of ZAP-70 expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

ZAP-70 (1E7.2): sc-32760 is recommended as a control antibody for monitoring of ZAP-70 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

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, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ZAP-70 gene expression knockdown using RT-PCR Primer: ZAP-70 (m)-PR: sc-36867-PR (20 μ l, 413 bp). Annealing temperature for the primers should be $55-60^{\circ}\text{C}$ and the extension temperature should be $68-72^{\circ}\text{C}$.

SELECT PRODUCT CITATIONS

1. Shen, Y., et al. 2019. Fas signaling-mediated TH9 cell differentiation favors bowel inflammation and antitumor functions. *Nat. Commun.* 10: 2924.

RESEARCH USE

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