

# ZAP-70 siRNA (h): sc-29526

## 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  $Ti$   $\alpha$  and  $\beta$  chains, the CD3  $\gamma$ ,  $\delta$  and  $\epsilon$  chains and a  $\zeta$ -containing homodimer or heterodimer. The disulfide-linked  $Ti$   $\alpha$ - $\beta$  heterodimer is responsible for antigen recognition, but the short 5 amino acid cytoplasmic domains of  $Ti$   $\alpha$  and  $\beta$  are unlikely to be sufficient to couple to intracellular signaling pathways. In contrast, the structured features of the CD3 and  $\zeta$  subunits suggest a role in signal transduction. Of these, the  $\zeta$  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  $\zeta$  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  $\zeta$  and  $\eta$  chains of the T cell receptor. *J. Biol. Chem.* 263: 9874-9878.
3. Baniyash, M., et al. 1988. The T cell antigen receptor  $\zeta$  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  $\zeta$  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.

## CHROMOSOMAL LOCATION

Genetic locus: ZAP70 (human) mapping to 2q11.2.

## PRODUCT

ZAP-70 siRNA (h) 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ZAP-70 shRNA Plasmid (h): sc-29526-SH and ZAP-70 shRNA (h) Lentiviral Particles: sc-29526-V as alternate gene silencing products.

For independent verification of ZAP-70 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-29526A, sc-29526B and sc-29526C.

## STORAGE AND RESUSPENSION

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

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

## APPLICATIONS

ZAP-70 siRNA (h) is recommended for the inhibition of ZAP-70 expression in human 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  $\mu$ M in 66  $\mu$ 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 (h)-PR: sc-29526-PR (20  $\mu$ l, 573 bp). Annealing temperature for the primers should be  $55-60^{\circ}$  C and the extension temperature should be  $68-72^{\circ}$  C.

## SELECT PRODUCT CITATIONS

1. Kim, K.C., et al. 2011. The effect of CD4 receptor downregulation and its downstream signaling molecules on HIV-1 latency. *Biochem. Biophys. Res. Commun.* 404: 646-651.

## RESEARCH USE

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

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.