SANTA CRUZ BIOTECHNOLOGY, INC.

CD3-ε siRNA (h): sc-29989

BACKGROUND

The T cell antigen receptor (TCR) recognizes foreign antigens and translates such recognition events into intracellular signals that elicit a change in the cell from a dormant to an activated state. Much of this signaling process can be attributed to a multisubunit complex of proteins that associates directly with the TCR. This complex has been designated CD3 (cluster of differentiation 3). It is composed of five invariant polypeptide chains that associate to form three dimers: a heterodimer of γ and ε chains (γε), a heterodimer of δ and ε chains (δε) and a homodimer of two ζ chains (ζζ) or a heterodimer of ζ and η chains (ζη). The ζ and η chains are encoded by the same gene but differ in their carboxyl-terminal ends due to an alternative splicing event. The γ, ε and δ chains each contain a single copy of a conserved immunoreceptor tyrosine-based activation motif (ITAM). In contrast, the ζ chain contains three consecutive copies of the same motif. Phosphorylated ITAMs act as docking sites for protein kinases such as ZAP-70 and Syk and are also capable of regulating their kinase activity. The crystal structure of the ZAP-70 SH2 domains bound to the ζ chain ITAMs has been solved.

REFERENCES

1. Exley, M., et al. 1991. Structure, assembly and intracellular transport of the T cell receptor for antigen. Semin. Immunol. 3: 283-297.
2. Weiss, A., et al. 1991. Signal transduction by the T cell antigen receptor. Prog. Immunol. 3: 313-324.
3. Chan, A.C., et al. 1994. The role of protein tyrosine kinases and protein tyrosine phosphatases in cell antigen receptor signal transduction. Annu. Rev. Immunol. 12: 555-592.
4. Aoe, T., et al. 1994. Different cytoplasmic structure of the CD3 ζ family dimer modulates the activation signal and function of T cells. Int. Immunol. 6: 1671-1679.
5. Ohno, H., et al. 1994. Targeted disruption of the CD3 η locus causes high lethality in mice: modulation of Oct-1 transcription on the opposite strand. EMBO J. 13: 1157-1165.
6. Neumeister, E.N., et al. 1995. Binding of ZAP-70 to phosphorylated T-cell receptor ζ and ε enhances its autophosphorylation and generates specific binding sites for SH2 domain-containing proteins. Mol. Cell. Biol. 15: 3171-3178.

CHROMOSOMAL LOCATION

Genetic locus: CD3E (human) mapping to 11q23.3.

RPRODUCT

CD3-ε 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CD3-ε shRNA Plasmid (h): sc-29989-SH and CD3-ε shRNA (h) Lentiviral Particles: sc-29989-V as alternate gene silencing products.

For independent verification of CD3-ε (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-29989A, sc-29989B and sc-29989C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

CD3-ε shRNA (h) Lentiviral Particles is recommended for the inhibition of CD3-ε 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 µM in 66 µl. 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

CD3-ε (UCH-T1): sc-1179 is recommended as a control antibody for monitoring of CD3-ε 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 CD3-ε gene expression knockdown using RT-PCR Primer: CD3-ε (h)-PR: sc-29989-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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

PROTOCOLS

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