SANTA CRUZ BIOTECHNOLOGY, INC.

SIT siRNA (h): sc-45334

BACKGROUND

T lymphocytes express several low molecular mass transmembrane adaptor proteins that recruit SH2 domain-containing intracellular molecules to the cell membrane via tyrosine-based signaling pathways. One such protein, SIT (SHP2 interacting transmembrane adaptor protein) is a disulfide-linked homodimeric glycoprotein that is expressed in lymphocytes. SIT is reduced to half its molecular mass via endoglycosidase treatment. It contains five potential tyrosine phosphorylation sites, suggesting a role in TCR-mediated recruitment of SH2 domain-containing intracellular signaling molecules to the plasma membrane. SIT interacts with SHP2 and also with the adaptor protein GRB2. In addition, it is a substrate for the Src protein kinases Fyn, Lck and ZAP-70.

REFERENCES

1. Marie-Cardine, A., Kirchgessner, H., Bruyns, E., Shevchenko, A., Mann, M., Autschbach, F., Ratnofsky, S., Meuer, S. and Schraven, B. 1999. SHP2-interacting transmembrane adaptor protein (SIT), a novel disulfide-linked dimer regulating human T cell activation. J. Exp. Med. 189: 1181-1194.
2. Judd, B.A. and Koretzky, G.A. 2000. Antigen specific T lymphocyte activation. Rev. Immunogenet. 2: 164-174.
3. Zhang, W. and Samelson, L.E. 2000. The role of membrane-associated adaptors in T cell receptor signalling. Semin. Immunol. 12: 35-41.
4. Pfrepper, K.I., Marie-Cardine, A., Simeoni, L., Kuramitsu, Y., Leo, A., Spicka, J., Hilgert, I., Scherer, J. and Schraven, B. 2001. Structural and functional dissection of the cytoplasmic domain of the transmembrane adaptor protein SIT (SHP2-interacting transmembrane adaptor protein). Eur. J. Immunol. 31: 1825-1836.
5. LocusLink Report (LocusID: 27240). http://www.ncbi.nlm.nih.gov/LocusLink/

CHROMOSOMAL LOCATION

Genetic locus: SIT1 (human) mapping to 9p13.3.

PRODUCT

SIT 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 SIT shRNA Plasmid (h): sc-45334-SH and SIT shRNA (h) Lentiviral Particles: sc-45334-V as alternate gene silencing products.

For independent verification of SIT (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-45334A, sc-45334B and sc-45334C.

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.

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

SIT siRNA (h) is recommended for the inhibition of SIT 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

SIT (F-9): sc-271933 is recommended as a control antibody for monitoring of SIT 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 SIT gene expression knockdown using RT-PCR Primer: SIT (h)-PR: sc-45334-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.