SANTA CRUZ BIOTECHNOLOGY, INC.

SPEC1 siRNA (h): sc-78876

BACKGROUND

Rho GTPases are molecular switches that regulate many essential cellular processes, including Actin dynamics, cell adhesion, cell-cycle progression and transcription. Cdc42, a small GTPase, regulates Actin polymerization, elongation of cell shape and cell signaling through interactions with many different downstream effector proteins, most of which contain a Cdc42-binding motif known as a CRIB domain. SPEC1 (small protein effector 1 of Cdc42), also known as CDC42SE1 (CDC42 small effector 1) or SCIP1, is a 79 amino acid cytoplasmic protein that may participate in the organization of the Actin cytoskeleton by acting downstream of Cdc42, thereby inducing Actin filament assembly and inducing cell shape changes. SPEC1 may also be involved in Cdc42-mediated F-Actin accumulation at the immunological synapse of activated T cells and may function in early contractile events during phagocytosis in macrophages. Containing a CRIB domain and a member of the Cdc42SE/SPEC family, SPEC1 is widely expressed and exists as two alternatively spliced isoforms.

REFERENCES

1. Hall, A. 1992. Ras-related GTPases and the cytoskeleton. Mol. Biol. Cell 3: 475-479.
2. Nobes, C.D., et al. 1995. Rho, Rac, and Cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with Actin stress fibers, lamellipodia, and filopodia. Cell 81: 53-62.
3. Olson, M.F., et al. 1995. An essential role for Rho, Rac, and Cdc42 GTPases in cell cycle progression through G_1. Science 269: 1270-1272.
4. Pirone, D.M., et al. 2000. SPECs, small binding proteins for Cdc42. J. Biol. Chem. 275: 22650-22656.
5. Pirone, D.M., et al. 2001. The genomic structure of the human SPEC1 gene reveals complex splicing and close promoter proximity to the AF1q translocation gene. Gene 273: 295-303.

CHROMOSOMAL LOCATION

Genetic locus: CDC42SE1 (human) mapping to 1q21.3.

PRODUCT

SPEC1 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 SPEC1 shRNA Plasmid (h): sc-78876-SH and SPEC1 shRNA (h) Lentiviral Particles: sc-78876-V as alternate gene silencing products.

For independent verification of SPEC1 (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-78876A, sc-78876B and sc-78876C.

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

SPEC1 siRNA (h) is recommended for the inhibition of SPEC1 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

SPEC1 (E-2): sc-514978 is recommended as a control antibody for monitoring of SPEC1 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 SPEC1 gene expression knockdown using RT-PCR Primer: SPEC1 (h)-PR: sc-78876-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.