

SPRED2 siRNA (h): sc-94969

BACKGROUND

SPRED2 (Sprouty-related, EVH1 domain-containing protein 2) is a 418 amino acid protein that localizes to the peripheral membrane and contains one WH1 domain, one Sprouty domain and one KBD domain. Expressed in prostate, skin, liver, salivary gland and small intestine, SPRED2 exists as a homodimer or a heterodimer (with SPRED1) that functions as a tyrosine kinase substrate and acts to inhibit growth-factor-induced MAP kinase (ERK 2) cascade activation. Human SPRED2 is subject to phosphorylation on Tyr 228 or Tyr 231, an event that leads to the ubiquitination and subsequent degradation of SPRED2 by the proteasome. Abnormal expression of SPRED2 is associated with a variety of malignant tumors, suggesting a role for SPRED2 in carcinogenesis. Additionally, disruption of the gene encoding SPRED2 that leads to an activation of the ERK 2 pathway may cause dwarfism.

REFERENCES

1. Wakioka, T., et al. 2001. SPRED is a Sprouty-related suppressor of Ras signalling. *Nature* 412: 647-651.
2. Engelhardt, C.M., et al. 2004. Expression and subcellular localization of SPRED proteins in mouse and human tissues. *Histochem. Cell Biol.* 122: 527-538.
3. Nonami, A., et al. 2004. SPRED1 negatively regulates interleukin-3-mediated ERK/mitogen-activated protein (MAP) kinase activation in hematopoietic cells. *J. Biol. Chem.* 279: 52543-52551.
4. Nobuhisa, I., et al. 2004. SPRED2 suppresses aorta-gonad-mesonephros hematopoiesis by inhibiting MAP kinase activation. *J. Exp. Med.* 199: 737-742.
5. Miyoshi, K., et al. 2004. The Sprouty-related protein, Spred, inhibits cell motility, metastasis, and Rho-mediated Actin reorganization. *Oncogene* 23: 5567-5576.
6. King, J.A., et al. 2005. Distinct requirements for the Sprouty domain for functional activity of SPRED proteins. *Biochem. J.* 388: 445-454.

CHROMOSOMAL LOCATION

Genetic locus: SPRED2 (human) mapping to 2p14.

PRODUCT

SPRED2 siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see SPRED2 shRNA Plasmid (h): sc-94969-SH and SPRED2 shRNA (h) Lentiviral Particles: sc-94969-V as alternate gene silencing products.

For independent verification of SPRED2 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3 nmol of lyophilized siRNA. These include: sc-94969A, sc-94969B and sc-94969C.

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 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

SPRED2 siRNA (h) is recommended for the inhibition of SPRED2 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 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

SPRED2 (6G8): sc-517018 is recommended as a control antibody for monitoring of SPRED2 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 SPRED2 gene expression knockdown using RT-PCR Primer: SPRED2 (h)-PR: sc-94969-PR (20 μ l). 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.