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SANTA CRUZ BIOTECHNOLOGY, INC.

SPRED1 siRNA (m): sc-153783

BACKGROUND

SPRED1 (sprouty-related, EVH1 domain containing 1), also known as NFLS, is a 444 amino acid protein that contains one KBD domain, one sprouty domain and one WH1 domain. Localized to the cell membrane and to cholesterol-rich membrane raft fractions, SPRED1 functions as a tyrosine kinase that regulates the activity of the ERK (also known as MAP kinase) cascade by inhibiting the growth-factor-mediated activation of ERK. SPRED1 can act independently as a homodimer or can function as a heterodimer with SPRED2 and, in addition to its ability to regulate ERK, is thought to negatively regulate the development of blood cells in bone marrow. Defects in the gene encoding SPRED1 are the cause of neurofibromatosis type 1-like syndrome (NFLS), an autosomal dominant disease that is characterized by multiple cafe-au-lait spots, axillary freckling and macrocephaly.

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. Spred-1 negatively regulates interleukin-3-mediated ERK/mitogen-activated protein (MAP) kinase activation in hematopoietic cells. J. Biol. Chem. 279: 52543-52551.
4. King, J.A., et al. 2005. Distinct requirements for the Sprouty domain for functional activity of Spred proteins. Biochem. J. 388: 445-454.
5. Nonami, A., et al. 2005. The Sprouty-related protein, Spred-1, localizes in a lipid raft/caveola and inhibits ERK activation in collaboration with caveolin-1. Genes Cells 10: 887-895.
6. Yoshida, T., et al. 2006. Spreds, inhibitors of the Ras/ERK signal transduction, are dysregulated in human hepatocellular carcinoma and linked to the malignant phenotype of tumors. Oncogene 25: 6056-6066.
7. Brems, H., et al. 2007. Germline loss-of-function mutations in SPRED1 cause a neurofibromatosis 1-like phenotype. Nat. Genet. 39: 1120-1126.

CHROMOSOMAL LOCATION

Genetic locus: Spred1 (mouse) mapping to 2 E5.

PRODUCT

SPRED1 siRNA (m) 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 SPRED1 shRNA Plasmid (m): sc-153783-SH and SPRED1 shRNA (m) Lentiviral Particles: sc-153783-V as alternate gene silencing products.

For independent verification of SPRED1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-153783A, sc-153783B and sc-153783C.

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

SPRED1 siRNA (m) is recommended for the inhibition of SPRED1 expression in mouse 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

SPRED1 (M23-P2G3): sc-101392 is recommended as a control antibody for monitoring of SPRED1 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 SPRED1 gene expression knockdown using RT-PCR Primer: SPRED1 (m)-PR: sc-153783-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.