

SPFH1 siRNA (h): sc-90434

BACKGROUND

SPFH1 (stomatin-prohibitin-flotillin-HflC/K domain-containing protein 1), also known as ERLIN1 (ER lipid raft associated 1), KE04 or KE04, is a 346 amino acid endoplasmic reticulum (ER) membrane protein that belongs to the band 7/ mec-2 family and forms a heteromeric complex with SPFH2. The SPFH1/ SPFH2 complex is a ring-shaped complex that mediates the ER-associated degradation (ERAD) of inositol 1,4,5-trisphosphate receptors (IP3Rs). SPFH1 is believed to be a component of lipid rafts and is expressed in the heart, placenta, liver, kidney, pancreas, prostate, testis, ovary and small intestine. The SPFH1 gene maps to human chromosome 10q24.31 and contains four transcripts. Human chromosome 10 comprises over 131 million base pairs which represent 99.4% of the euchromatic DNA, and includes one megabase of heterochromatic sequence within the pericentromeric region of the short and long arm of the chromosome. 1,357 genes are present, including 816 that are protein coding, and 430 that are pseudogenes.

REFERENCES

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3. Browman, D.T., et al. 2006. Erlin-1 and erlin-2 are novel members of the prohibitin family of proteins that define lipid-raft-like domains of the ER. *J. Cell Sci.* 119: 3149-3160.
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5. Wang, Y., et al. 2009. SPFH1 and SPFH2 mediate the ubiquitination and degradation of inositol 1,4,5-trisphosphate receptors in muscarinic receptor-expressing HeLa cells. *Biochim. Biophys. Acta* 1793: 1710-1718.
6. Wojcikiewicz, R.J., et al. 2009. When worlds collide: IP₃ receptors and the ERAD pathway. *Cell Calcium* 46: 147-153.
7. Brodsky, J.L., et al. 2009. Substrate-specific mediators of ER associated degradation (ERAD). *Curr. Opin. Cell Biol.* 21: 516-521.

CHROMOSOMAL LOCATION

Genetic locus: ERLIN1 (human) mapping to 10q24.31.

PRODUCT

SPFH1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see SPFH1 shRNA Plasmid (h): sc-90434-SH and SPFH1 shRNA (h) Lentiviral Particles: sc-90434-V as alternate gene silencing products.

For independent verification of SPFH1 (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-90434A, sc-90434B and sc-90434C.

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

SPFH1 siRNA (h) is recommended for the inhibition of SPFH1 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

SPFH1 (A-7): sc-514820 is recommended as a control antibody for monitoring of SPFH1 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 SPFH1 gene expression knockdown using RT-PCR Primer: SPFH1 (h)-PR: sc-90434-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.

PROTOCOLS

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