

SPPL2a siRNA (h): sc-76570

BACKGROUND

Intramembrane proteolysis is now widely recognized as an important physiological pathway required for reverse signaling and membrane protein degradation. Aspartyl intramembrane cleaving proteases of the GXGD-type play an important regulatory role in health and disease. Signal peptide peptidase (SPP) and SPP-like (SPPL) peptidases belong to the family of GXGD-type aspartyl proteases. SPPL2a (signal peptide peptidase-like 2a), also known as IMP3 (intramembrane protease 3) or PSL2 (presenilin-like protein 2), is a 520 amino acid multi-pass membrane protein that contains one protease associated domain. SPPL2a functions as an intramembrane protease and may be involved in the processing of Fas-L, a type II transmembrane protein belonging to the tumor necrosis factor family.

REFERENCES

1. Friedmann, E., et al. 2004. Consensus analysis of signal peptide peptidase and homologous human aspartic proteases reveals opposite topology of catalytic domains compared with presenilins. *J. Biol. Chem.* 279: 50790-50798.
2. Krawitz, P., et al. 2005. Differential localization and identification of a critical aspartate suggest non-redundant proteolytic functions of the presenilin homologues SPPL2b and SPPL3. *J. Biol. Chem.* 280: 39515-39523.
3. Friedmann, E., et al. 2006. SPPL2a and SPPL2b promote intramembrane proteolysis of TNF α in activated dendritic cells to trigger IL-12 production. *Nat. Cell Biol.* 8: 843-848.
4. Kirkin, V., et al. 2007. The Fas ligand intracellular domain is released by ADAM10 and SPPL2a cleavage in T-cells. *Cell Death Differ.* 14: 1678-1687.
5. Fluhrer, R., et al. 2007. Signal peptide peptidases and γ -secretase: cousins of the same protease family? *Neurodegener. Dis.* 4: 112-116.
6. Martin, L., et al. 2008. Regulated intramembrane proteolysis of Bri2 (Itm2b) by ADAM10 and SPPL2a/SPPL2b. *J. Biol. Chem.* 283: 1644-1652.
7. Martin, L., et al. 2009. Substrate requirements for SPPL2b-dependent regulated intramembrane proteolysis. *J. Biol. Chem.* 284: 5662-5670.

CHROMOSOMAL LOCATION

Genetic locus: SPPL2A (human) mapping to 15q21.2.

PRODUCT

SPPL2a 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 SPPL2a shRNA Plasmid (h): sc-76570-SH and SPPL2a shRNA (h) Lentiviral Particles: sc-76570-V as alternate gene silencing products.

For independent verification of SPPL2a (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-76570A, sc-76570B and sc-76570C.

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

SPPL2a siRNA (h) is recommended for the inhibition of SPPL2a 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

SPPL2a (1C7): sc-293375 is recommended as a control antibody for monitoring of SPPL2a 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 SPPL2a gene expression knockdown using RT-PCR Primer: SPPL2a (h)-PR: sc-76570-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.