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SPP shRNA (h) Lentiviral Particles: sc-45549-V

BACKGROUND

The endoplasmic reticulum exerts a quality control over newly synthesized proteins and a variety of components have been implicated in the specific recognition of aberrant or misfolded polypeptides. Signal peptide peptidase (SPP) catalyzes intramembrane proteolysis of some signal peptides after they have been cleaved from a preprotein, resulting in the release of the fragment from the ER membrane into the cytoplasm. SPP is required to generate lymphocyte cell surface (HLA-E) epitopes derived from MHC class I signal peptides, and may play a role in graft rejection. It also may be necessary for the removal of the signal peptide that remains attached to the hepatitis C virus core protein after the initial proteolytic processing of the polyprotein.

REFERENCES

1. Crawshaw, S.G., et al. 2004. A misassembled transmembrane domain of a polytopic protein associates with signal peptide peptidase. *Biochem. J.* 384: 9-17.
2. Nyborg, A.C., et al. 2004. A signal peptide peptidase (SPP) reporter activity assay based on the cleavage of type II membrane protein substrates provides further evidence for an inverted orientation of the SPP active site relative to presenilin. *J. Biol. Chem.* 279: 43148-43156.
3. 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.
4. Okamoto, K., et al. 2004. Intramembrane proteolysis and endoplasmic reticulum retention of hepatitis C virus core protein. *J. Virol.* 78: 6370-6380.
5. Casso, D.J., et al. 2005. *Drosophila* signal peptide peptidase is an essential protease for larval development. *Genetics* 170: 139-148.

CHROMOSOMAL LOCATION

Genetic locus: HM13 (human) mapping to 20q11.21.

PRODUCT

SPP shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see SPP siRNA (h): sc-45549 and SPP shRNA Plasmid (h): sc-45549-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

SPP shRNA (h) Lentiviral Particles is recommended for the inhibition of SPP expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

SPP (N-16): sc-34950 is recommended as a control antibody for monitoring of SPP 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor SPP gene expression knockdown using RT-PCR Primer: SPP (h)-PR: sc-45549-PR (20 μ l, 581 bp). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.