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PLSCR1 shRNA (h) Lentiviral Particles: sc-44028-V

BACKGROUND

The calcium-dependent plasma membrane protein phospholipid scramblase 1 (PLSCR1) contributes to the transbilayer movement of phosphatidylserine and other membrane phospholipids upon influx of calcium into the cytosol. This movement results in plasma membrane phospholipid remodelling and surface exposure of phosphatidylserine in injured or apoptotic cells, which leads to cell death. Interferons and other cytokines induce expression of PLSCR1, implying that PLSCR1 also functions in cytokine signaling pathways. EGF stimulation results in tyrosine phosphorylation of PLSCR1 on tyrosines 69 and 74, which allows it to interact with Shc, and thereby connecting Src kinase activation to stimulation of the EGF receptor.

REFERENCES

1. Wiedmer, T., et al. 2003. Palmitoylation of phospholipid scramblase 1 controls its distribution between nucleus and plasma membrane. *Biochemistry* 42: 1227-1233.
2. Rami, A., et al. 2003. Spatial resolution of phospholipid scramblase 1 (PLSCR1), caspase-3 activation and DNA-fragmentation in the human hippocampus after cerebral ischemia. *Neurochem. Int.* 43: 79-87.
3. Nanjundan, M., et al. 2003. Plasma membrane phospholipid scramblase 1 promotes EGF-dependent activation of c-Src through the epidermal growth factor receptor. *J. Biol. Chem.* 278: 37413-3748.
4. Chen, M.H., et al. 2004. Phospholipid scramblase 1 (PLSCR1) contains a non-classical nuclear localization signal with unique binding site in importin α . *J. Biol. Chem.* 280: 10599-10606.
5. Dong, B., et al. 2004. Phospholipid scramblase 1 potentiates the antiviral activity of interferon. *J. Virol.* 78: 8983-8993.

CHROMOSOMAL LOCATION

Genetic locus: PLSCR1 (human) mapping to 3q24.

PRODUCT

PLSCR1 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 PLSCR1 siRNA (h): sc-44028 and PLSCR1 shRNA Plasmid (h): sc-44028-SH as alternate gene silencing products.

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.

APPLICATIONS

PLSCR1 shRNA (h) Lentiviral Particles is recommended for the inhibition of PLSCR1 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

PLSCR1 (N-17): sc-27779 is recommended as a control antibody for monitoring of PLSCR1 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 PLSCR1 gene expression knockdown using RT-PCR Primer: PLSCR1 (h)-PR: sc-44028-PR (20 μ l, 484 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

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.