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caveolin-1 shRNA (h2) Lentiviral Particles: sc-44202-V

BACKGROUND

Caveolae (also known as plasmalemmal vesicles) are 50-100 nM flask-shaped membranes that represent a subcompartment of the plasma membrane. On the basis of morphological studies, caveolae have been implicated to function in the transcytosis of various macromolecules (including LDL) across capillary endothelial cells, the uptake of small molecules via potocytosis and the compartmentalization of certain signaling molecules, including G protein-coupled receptors. Three proteins, caveolin-1, caveolin-2 and caveolin-3, have been identified as principal components of caveolae. Two forms of caveolin-1, designated α and β , share a distinct but overlapping cellular distribution and differ by an amino-terminal 31 amino acid sequence which is absent from the β isoform. Caveolin-1 shares 31% identity with caveolin-2 and 65% identity with caveolin-3 at the amino acid level. Functionally, the three proteins differ in their interactions with heterotrimeric G protein isoforms.

REFERENCES

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2. Rothberg, K.G., et al. 1992. Caveolin, a protein component of caveolae membrane coats. *Cell* 68: 673-682.
3. Lisanti, M.P., et al. 1994. Characterization of caveolin-rich membrane domains isolated from an endothelial-rich source: implications for human disease. *J. Cell Biol.* 126: 111-126.
4. Zurzolo, C., et al. 1994. VIP21/caveolin, glycosphingolipid clusters and the sorting of glycosylphosphatidylinositol-anchored proteins in epithelial cells. *EMBO J.* 13: 42-53.
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CHROMOSOMAL LOCATION

Genetic locus: CAV1 (human) mapping to 7q31.2.

PRODUCT

caveolin-1 shRNA (h2) 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 caveolin-1 siRNA (h2): sc-44202 and caveolin-1 shRNA Plasmid (h2): sc-44202-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.

APPLICATIONS

caveolin-1 shRNA (h2) Lentiviral Particles is recommended for the inhibition of caveolin-1 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 lentiviral transducing particles per milliliter; contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

caveolin-1 (N-20): sc-894 is recommended as a control antibody for monitoring of caveolin-1 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 goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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 goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor caveolin-1 gene expression knockdown using RT-PCR Primer: caveolin-1 (h2)-PR: sc-44202-PR (20 μ l, 464 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.

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