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EDG-6 shRNA (h) Lentiviral Particles: sc-43747-V

BACKGROUND

The EDG (endothelial differentiation gene) family of G protein-coupled receptors consists of eight family members that bind lysophospholipid (LPL) mediators, including sphingosine-1-phosphate (SPP) and lysophosphatidic acid (LPA). EDG-1, EDG-3, EDG-5 (also designated H218 and AGR16) and EDG-8 bind SPP with high affinity. EDG-6 is a low affinity receptor for SPP. LPA preferentially binds to EDG-2, EDG-4 and EDG-7. The EDG receptors couple to multiple G proteins to signal through Ras, MAP kinase, Rho, Phospholipase C or other tyrosine kinases, which lead to cell survival, growth, migration and differentiation. EDG-1 signals through G_i proteins to activate Akt and is expressed in glioma cells. EDG-2 is expressed in brain, especially in white matter tract regions, while EDG-3 is expressed in cardiovascular tissue and in cerebellum. EDG-4 is highly expressed on leukocytes and brain, and EDG-5 has wide tissue distribution, including cardiovascular tissue and brain. EDG-6, which is expressed in lymphoid and hematopoietic tissues and in lung, signals through G_{1/0} proteins, which activate growth related pathways.

REFERENCES

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- Van Brocklyn, J.R., et al. 2000. Sphingosine-1-phosphate is a ligand for the G protein-coupled receptor EDG-6. *Blood* 95: 2624-2629.
- Pyne, S., et al. 2000. Sphingosine 1-phosphate signalling in mammalian cells. *Biochem. J.* 349: 385-402.
- Sato, K., et al. 2000. Differential roles of Edg-1 and Edg-5, sphingosine 1-phosphate receptors, in the signaling pathways in C6 glioma cells. *Brain Res. Mol. Brain Res.* 85: 151-160.
- Morales-Ruiz, M., et al. 2001. Sphingosine-1-phosphate activates Akt, nitric oxide production and chemotaxis through a G_i-protein/ phosphoinositide 3-kinase pathway in endothelial cells. *J. Biol. Chem.* 276: 19672-19677.
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CHROMOSOMAL LOCATION

Genetic locus: S1PR4 (human) mapping to 19p13.3.

PRODUCT

EDG-6 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 2 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10⁶ 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 EDG-6 siRNA (h): sc-43747 and EDG-6 shRNA Plasmid (h): sc-43747-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

EDG-6 shRNA (h) Lentiviral Particles is recommended for the inhibition of EDG-6 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ 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

EDG-6 (1) : sc-65216 is recommended as a control antibody for monitoring of EDG-6 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-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor EDG-6 gene expression knockdown using RT-PCR Primer: EDG-6 (h)-PR: sc-43747-PR (20 µl, 459 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.

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.

PROTOCOLS

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