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EG-VEGF shRNA (h) Lentiviral Particles: sc-45392-V

BACKGROUND

Endocrine gland-derived vascular endothelial growth factor (EG-VEGF) induces proliferation, migration and fenestration in capillary endothelial cells derived from endocrine glands. EG-VEGF possesses a HIF-1 binding site; its expression is induced by hypoxia and restricted to the steroidogenic glands (ovary, testis, adrenal and placenta). EG-VEGF expression is often complementary to the expression of VEGF, suggesting that these molecules function in a coordinated manner. EG-VEGF is an example of a class of highly specific mitogens that act to regulate proliferation and differentiation of the vascular endothelium in a tissue-specific manner. EG-VEGF is expressed largely in one type of tissue and acts selectively on one type of endothelium. EG-VEGF, possibly through binding to a G protein-coupled receptor, results in the activation of MAPK p44/42 and phosphatidylinositol 3-kinase signaling pathways, leading to proliferation, migration and survival of responsive endothelial cells.

REFERENCES

1. Carmeliet, P. 2001. Cardiovascular biology. Creating unique blood vessels. *Nature* 412: 868-869.
2. LeCouter, J., et al. 2001. Identification of an angiogenic mitogen selective for endocrine gland endothelium. *Nature* 412: 877-984.
3. Lin, R., et al. 2002. Characterization of endocrine gland-derived vascular endothelial growth factor signaling in adrenal cortex capillary endothelial cells. *J. Biol. Chem.* 277: 8724-8729.
4. Lin, D.C., et al. 2002. Identification and molecular characterization of two closely related G protein-coupled receptors activated by prokineticins/EG-VEGF. *J. Biol. Chem.* 277: 19276-19280.
5. Ferrara, N., et al. 2002. Endocrine gland vascular endothelial growth factor (EG-VEGF) and the hypothesis of tissue-specific regulation of angiogenesis. *Endocr. Res.* 28: 763-764.
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CHROMOSOMAL LOCATION

Genetic locus: PROK1 (human) mapping to 1p21.

PRODUCT

EG-VEGF 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 EG-VEGF siRNA (h): sc-45392 and EG-VEGF shRNA Plasmid (h): sc-45392-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

EG-VEGF shRNA (h) Lentiviral Particles is recommended for the inhibition of EG-VEGF 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

EG-VEGF (A-12): sc-30343 is recommended as a control antibody for monitoring of EG-VEGF 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 EG-VEGF gene expression knockdown using RT-PCR Primer: EG-VEGF (h)-PR: sc-45392-PR (20 μ l). 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.

PROTOCOLS

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