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ET-1 shRNA (h) Lentiviral Particles: sc-45394-V

BACKGROUND

The human endothelins represent a gene family comprised of endothelin-1, endothelin-2 and endothelin-3, also known as ET-1, ET-2 and ET-3. Endothelins can affect the central nervous system and neuronal excitability, and they elicit potent vasoconstrictor action. The two receptor subtypes responsible for inducing vasoconstriction and vasodilation, ETA and ETB, have different receptor affinities for ET-1, ET-2 and ET-3. Of the three isopeptides, ET-2 has the most potent vasoconstrictor activity. Biologically active ETs are proteolytically generated from a larger precursor, the big-endothelin, by action of the endothelin-converting enzyme (ECE) family. ET-1 is a potent, 21 amino acid vasoconstrictor peptide produced by vascular endothelial cells. The ET-2 cDNA is 1.3 kb in length and encodes a proprotein consisting of 178 amino acid residues. ET3 mRNA encodes a 230 amino acid precursor that includes ET3 and a 15 amino acid homologous segment called the ET3-like sequence.

REFERENCES

1. Itoh, Y., et al. 1988. Cloning and sequence analysis of cDNA encoding the precursor of a human endothelium-derived vasoconstrictor peptide, endothelin: identity of human and porcine endothelin. *FEBS Lett.* 231: 440-444.
2. Masaki, T. 1989. The discovery, the present state, and the future prospects of endothelin. *J. Cardiovasc. Pharmacol.* 13: S1-S4, S18.
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4. Godfraind, T., et al. 1989. Effect of endothelin-1 on calcium channel gating by agonists in vascular smooth muscle. *J. Cardiovasc. Pharmacol.* 13: S112-117, S123.
5. Arinami, T., et al. 1991. Chromosomal assignments of the human endothelin family genes: the endothelin-1 gene (EDN1) to 6p23-p24, the endothelin-2 gene (EDN2) to 1p34, and the endothelin-3 gene (EDN3) to 20q13.2-q13.3. *Am. J. Hum. Genet.* 48: 990-996.

CHROMOSOMAL LOCATION

Genetic locus: EDN1 (human) mapping to 6p24.1.

PRODUCT

ET-1 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 ET-1 siRNA (h): sc-45394 and ET-1 shRNA Plasmid (h): sc-45394-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

ET-1 shRNA (h) Lentiviral Particles is recommended for the inhibition of ET-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 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

ET-1 (N-8): sc-21625 is recommended as a control antibody for monitoring of ET-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 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 ET-1 gene expression knockdown using RT-PCR Primer: ET-1 (h)-PR: sc-45394-PR (20 μ l, 586 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.

PROTOCOLS

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