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ECE-1 shRNA (m) Lentiviral Particles: sc-44479-V

BACKGROUND

Endothelin converting enzymes (ECE-1 and ECE-2) are type II metalloproteases that convert big endothelin-1 to vasoactive endothelin-1. Both ECE-1 and ECE-2 belong to the peptidase family M13 and are Type II membrane proteins. There are several different isoforms of each ECE protein and the expression of the protein may therefore vary. All isoforms are expressed in umbilical endothelial cells, atrium cardiomyocytes and ventricles, polynuclear neutrophils and fibroblasts. Endothelin-converting enzyme-1 (ECE-1) converts big endothelin-1 to endothelin-1 by catalyzing the cleavage of the trp21-val22 bond in the precursor. The ECE-1 gene produces four isoforms from alternate promoters. The isoforms share the same extracellular catalytic domain and contain unique cytosolic tails, which results in their specific subcellular targeting.

REFERENCES

- Schmidt, M., et al. 1994. Molecular characterization of human and bovine endothelin converting enzyme (ECE-1). *FEBS Lett.* 356: 238-243.
- Ikeda, S., et al. 2002. Molecular isolation and characterization of novel four subisoforms of ECE-2. *Biochem. Biophys. Res. Commun.* 293: 421-426.
- Muller, L., et al. 2003. Heterodimerization of endothelin-converting enzyme-1 isoforms regulates the subcellular distribution of this metalloprotease. *J. Biol. Chem.* 278: 545-555.
- Mzhavia, N., et al. 2003. Characterization of endothelin-converting enzyme-2. Implication for a role in the nonclassical processing of regulatory peptides. *J. Biol. Chem.* 278: 14704-14711.

CHROMOSOMAL LOCATION

Genetic locus: Ece1 (mouse) mapping to 4 D3.

PRODUCT

ECE-1 shRNA (m) 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 ECE-1 siRNA (m): sc-44479 and ECE-1 shRNA Plasmid (m): sc-44479-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

ECE-1 shRNA (m) Lentiviral Particles is recommended for the inhibition of ECE-1 expression in mouse 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

ECE-1 (H-60): sc-25841 is recommended as a control antibody for monitoring of ECE-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 ECE-1 gene expression knockdown using RT-PCR Primer: ECE-1 (m)-PR: sc-44479-PR (20 μ l, 470 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.