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ECE-1 siRNA (h): sc-44478

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 Trp 21-Val 22 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., Kroger, B., Jacob, E., Seulberger, H., Subkowski, T., Otter, R., Meyer, T., Schmalzing, G. and Hillen, H. 1994. Molecular characterization of human and bovine endothelin converting enzyme (ECE-1). *FEBS Lett.* 356: 238-243.
- Ikeda, S., Emoto, N., Alimsardjono, H., Yokoyama, M. and Matsuo, M. 2002. Molecular isolation and characterization of novel four subisoforms of ECE-2. *Biochem. Biophys. Res. Commun.* 293: 421-426.
- Muller, L., Barret, A., Etienne, E., Meidan, R., Valdenaire, O., Corvol, P. and Tougaard, C. 2003. Heterodimerization of endothelin-converting enzyme-1 isoforms regulates the subcellular distribution of this metalloprotease. *J. Biol. Chem.* 278: 545-555.
- Mzhavia, N., Pan, H., Che, F.Y., Fricker, L.D. and Devi, L.A. 2003. Characterization of endothelin-converting enzyme-2. Implication for a role in the nonclassical processing of regulatory peptides. *J. Biol. Chem.* 278: 14704-14711.
- LocusLink Report (LocusID: 1889). <http://www.ncbi.nlm.nih.gov/LocusLink/>

CHROMOSOMAL LOCATION

Genetic locus: ECE1 (human) mapping to 1p36.12.

PRODUCT

ECE-1 siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ECE-1 shRNA Plasmid (h): sc-44478-SH and ECE-1 shRNA (h) Lentiviral Particles: sc-44478-V as alternate gene silencing products.

For independent verification of ECE-1 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44478A, sc-44478B and sc-44478C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

ECE-1 siRNA (h) is recommended for the inhibition of ECE-1 expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

ECE-1 (A-6): sc-376017 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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ECE-1 gene expression knockdown using RT-PCR Primer: ECE-1 (h)-PR: sc-44478-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.