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ET-2 siRNA (h): sc-45396

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. The human endothelin-1, 2 and 3 genes (EDN1, EDN2, and EDN3) map to chromosome 6p24, 1p34.2, and 20q13, respectively. 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. Inoue, A., Yanagisawa, M., Takawa, Y., Mitsui, Y., Kobayashi, M. and Masaki, T. 1989. The human preproendothelin-1 gene. Complete nucleotide sequence and regulation of expression. *J. Biol. Chem.* 264: 14954-14959.
2. Arinami, T., Ishikawa, M., Inoue, A., Yanagisawa, M., Masaki, T., Yoshida, M.C. and Hamaguchi, H. 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.
3. Nguyen, B.N. and Johnson, J.A. 1998. The role of endothelin in heart failure and hypertension. *Pharmacotherapy* 18: 706-719.
4. Online Mendelian Inheritance in Man, OMIM[™]. 1998. Johns Hopkins University, Baltimore, MD. MIM Number: 131241. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>

CHROMOSOMAL LOCATION

Genetic locus: EDN2 (human) mapping to 1p34.2.

PRODUCT

ET-2 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 ET-2 shRNA Plasmid (h): sc-45396-SH and ET-2 shRNA (h) Lentiviral Particles: sc-45396-V as alternate gene silencing products.

For independent verification of ET-2 (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-45396A, sc-45396B and sc-45396C.

RESEARCH USE

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

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

ET-2 siRNA (h) is recommended for the inhibition of ET-2 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

ET-2 (3B4-1C5): sc-293248 is recommended as a control antibody for monitoring of ET-2 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 ET-2 gene expression knockdown using RT-PCR Primer: ET-2 (h)-PR: sc-45396-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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