SANTA CRUZ BIOTECHNOLOGY, INC.

ET-1 siRNA (h): sc-45394

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.
3. Watanabe, T., et al. 1989. Positive inotropic and vasoconstrictive effects of endothelin-1 in *in vivo* and *in vitro* experiments: characteristics and the role of L-type calcium channels. J. Cardiovasc. Pharmacol. 13: S108-S111.

CHROMOSOMAL LOCATION

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

PRODUCT

ET-1 siRNA (h) is a pool of 2 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ET-1 shRNA Plasmid (h): sc-45394-SH and ET-1 shRNA (h) Lentiviral Particles: sc-45394-V as alternate gene silencing products.

For independent verification of ET-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-45394A and sc-45394B.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

ET-1 siRNA (h) is recommended for the inhibition of ET-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 µM in 66 µl. 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-1 (C7): sc-517436 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 reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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-1 gene expression knockdown using RT-PCR Primer: ET-1 (h)-PR: sc-45394-PR (20 µl, 598 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Marion, A., et al. 2012. Calpain-6 is an endothelin-1 signaling dependent protective factor in chemoresistant osteosarcoma. Int. J. Cancer 130: 2514-2525.
2. He, C., et al. 2013. Angiotensin II induces endothelin-1 expression in human hepatic stellate cells. Dig. Dis. Sci. 58: 2542-2549.
3. Gien, J., et al. 2013. Endothelin-1 impairs angiogenesis *in vitro* through Rho-kinase activation after chronic intrauterine pulmonary hypertension in fetal sheep. Pediatr. Res. 73: 252-262.

RESEARCH USE

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