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ET-3 siRNA (h): sc-45397

BACKGROUND

The endothelin (ET) family of proteins are vasoactive peptides that are involved in various functions throughout the body. ET-3 (endothelin 3), also known as EDN3, is a 238 amino acid secreted protein that belongs to the endothelin family and is essential for proper neuronal development. Expressed in placental stem villi vessels and in trophoblasts, ET-3 functions as a ligand for endothelin receptor type B (ETBR) and, through this interaction, mediates the maturation of enteric neurons and melanocytes. Although ET-3 is expressed as a 238 amino acid peptide, it is post-translationally modified to produce a short active isoform and a long inactive isoform. Defects in the gene encoding ET-3 are the cause of a variety of disorders, including Hirschsprung disease type 1 (HSCR1), congenital central hypoventilation syndrome (CCHS) and Waardenburg syndrome type IV (WS4).

REFERENCES

- Hofstra, R.M., et al. 1996. A homozygous mutation in the endothelin-3 gene associated with a combined Waardenburg type 2 and Hirschsprung phenotype (Shah-Waardenburg syndrome). *Nat. Genet.* 12: 445-447.
- Bolk, S., et al. 1996. Endothelin-3 frameshift mutation in congenital central hypoventilation syndrome. *Nat. Genet.* 13: 395-396.
- Bidaud, C., et al. 1997. Endothelin-3 gene mutations in isolated and syndromic Hirschsprung disease. *Eur. J. Hum. Genet.* 5: 247-251.
- Dupin, E., et al. 2000. Endothelin 3 induces the reversion of melanocytes to glia through a neural crest-derived glial-melanocytic progenitor. *Proc. Natl. Acad. Sci. USA* 97: 7882-7887.
- Pingault, V., et al. 2001. A heterozygous endothelin 3 mutation in Waardenburg-Hirschsprung disease: is there a dosage effect of EDN3/EDNRB gene mutations on neurocristopathy phenotypes? *J. Med. Genet.* 38: 205-209.

CHROMOSOMAL LOCATION

Genetic locus: EDN3 (human) mapping to 20q13.32.

PRODUCT

ET-3 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-3 shRNA Plasmid (h): sc-45397-SH and ET-3 shRNA (h) Lentiviral Particles: sc-45397-V as alternate gene silencing products.

For independent verification of ET-3 (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-45397A, sc-45397B and sc-45397C.

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

ET-3 siRNA (h) is recommended for the inhibition of ET-3 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-3 (5E8): sc-81944 is recommended as a control antibody for monitoring of ET-3 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-3 gene expression knockdown using RT-PCR Primer: ET-3 (h)-PR: sc-45397-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.