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## Produktinformation



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# ET-3 shRNA (h) Lentiviral Particles: sc-45397-V

## 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.
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- 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.
- Al-Jiffry, B.O., et al. 2002. Endothelin-3 induces both human and opossum gallbladder contraction mediated mainly by endothelin-B receptor subtype *in vitro*. *J. Gastroenterol. Hepatol.* 17: 324-331.

## CHROMOSOMAL LOCATION

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

## PRODUCT

ET-3 shRNA (h) 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  $\mu$ l frozen stock containing  $1.0 \times 10^6$  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 ET-3 siRNA (h): sc-45397 and ET-3 shRNA Plasmid (h): sc-45397-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^{\circ}$  C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^{\circ}$  C for up to one week. Avoid repeated freeze thaw cycles.

## APPLICATIONS

ET-3 shRNA (h) Lentiviral Particles is recommended for the inhibition of ET-3 expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  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

ET-3 (C-6): sc-21628 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## 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  $\mu$ l). Annealing temperature for the primers should be  $55-60^{\circ}$  C and the extension temperature should be  $68-72^{\circ}$  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.

## 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.

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