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TRPV3 shRNA (h) Lentiviral Particles: sc-44170-V

BACKGROUND

Transient receptor potential (TRP) proteins are cation-sensitive channels that modulate a myriad of cellular functions, including temperature sensation and vasoregulation. Transcribed from a gene adjacent to VR-1, the thermal-sensitive, Capsaicin-insensitive TRPV3 is expressed at warm temperatures; expression increases in response to noxious temperatures. Human TRPV3 is expressed in skin, tongue, dorsal root ganglion, trigeminal ganglion, spinal cord and brain. In addition, TRPV3 is co-expressed in dorsal root ganglion neurons with VR-1. TRPV3 associates with VR-1 and may modulate VR-1 activity. The 729 amino acid TRPV5 (ECAC1) protein comprises 6 transmembrane domains, multiple potential phosphorylation sites, an N-linked glycosylation site and three ankyrin repeat regions. It is abundantly expressed in kidney, jejunum and pancreas, and at lower levels in testis, prostate, placenta, brain, colon and rectum. TRPV5 controls the rate-limiting step of vitamin D3-regulated Ca²⁺ reabsorption in kidney and intestine; the 5'-flanking region of TRPV5 contains four putative vitamin D3-responsive elements.

REFERENCES

1. Smith, G.D., et al. 2000. TRPV3 is a temperature-sensitive vanilloid receptor-like protein. *Nature* 418: 186-190.
2. Muller, D., et al. 2000. Gene structure and chromosomal mapping of human epithelial calcium channel. *Biochem. Biophys. Res. Commun.* 275: 47-52.
3. Xu, H., et al. 2002. TRPV3 is a calcium-permeable temperature-sensitive cation channel. *Nature* 418: 181-186.
4. Peier, A.M., et al. 2002. A heat-sensitive TRP channel expressed in keratinocytes. *Science* 296: 2046-2049.

CHROMOSOMAL LOCATION

Genetic locus: TRPV3 (human) mapping to 17p13.2.

PRODUCT

TRPV3 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 µl frozen stock containing 1.0 x 10⁶ lentiviral transducing particles per milliliter in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see TRPV3 siRNA (h): sc-44170 and TRPV3 shRNA Plasmid (h): sc-44170-SH as alternate gene silencing products.

STORAGE

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

PROTOCOLS

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

APPLICATIONS

TRPV3 shRNA (h) Lentiviral Particles is recommended for the inhibition of TRPV3 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ 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

TRPV3 (K-16): sc-23372 is recommended as a control antibody for monitoring of TRPV3 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 TRPV3 gene expression knockdown using RT-PCR Primer: TRPV3 (h)-PR: sc-44170-PR (20 µl, 596 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.