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TRPV6 shRNA (m) Lentiviral Particles: sc-44172-V

BACKGROUND

The transient receptor potential (TRP) protein family consists of a diverse group of cation channels functioning in a variety of homeostatic and regulatory pathways. Four subfamilies exist, based on channel domain homology, not activating stimuli: C type (canonical or classical), V type (vanilloid receptor related), M type (melastatin related) and P type (PKD). TRPV6 belongs to the V type subfamily, and it facilitates calcium entry across the plasma membrane in pancreas, placenta, and to a lesser extent stomach and kidney tissue. Furthermore, prostate cancer cells overexpress TRPV6, while benign prostate tissues do not express the protein, implying a role for TRPV6 in malignant growth.

REFERENCES

1. Nilius, B., et al. 2002. Fast and slow inactivation kinetics of the Ca²⁺ channels ECaC1 and ECaC2 (TRPV5 and TRPV6). Role of the intracellular loop located between transmembrane segments 2 and 3. *J. Biol. Chem.* 277: 30852-30858.
2. Hoenderop, J.G., et al. 2003. Homo- and heterotetrameric architecture of the epithelial Ca²⁺ channels TRPV5 and TRPV6. *EMBO J.* 22: 776-785.
3. Fixemer, T., et al. 2003. Expression of the Ca²⁺-selective cation channel TRPV6 in human prostate cancer: a novel prognostic marker for tumor progression. *Oncogene* 22: 7858-7861.
4. Birnbaumer, L., et al. 2003. A comparison of the genes coding for canonical TRP channels and their M, V and P relatives. *Cell Calcium* 33: 419-432.
5. Hirnet, D., et al. 2003. The TRPV6 gene, cDNA and protein. *Cell Calcium* 33: 509-518.

CHROMOSOMAL LOCATION

Genetic locus: *Trpv6* (mouse) mapping to 6 B2.1.

PRODUCT

TRPV6 shRNA (m) 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 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 TRPV6 siRNA (m): sc-44172 and TRPV6 shRNA Plasmid (m): sc-44172-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

TRPV6 shRNA (m) Lentiviral Particles is recommended for the inhibition of TRPV6 expression in mouse 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

TRPV6 (H-90): sc-28763 is recommended as a control antibody for monitoring of TRPV6 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 goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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 goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor TRPV6 gene expression knockdown using RT-PCR Primer: TRPV6 (m)-PR: sc-44172-PR (20 µl, 520 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.