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ANKTM1 shRNA (h) Lentiviral Particles: sc-44780-V

BACKGROUND

Transient receptor potential ion channels (TRPCs) are a superfamily of six transmembrane segment-spanning, gated cation channels. TRPC subtypes mediate store-operated Ca^{2+} entry, a process involving Ca^{2+} influx and replenishment of Ca^{2+} stores formerly emptied through the action of inositol 1,4,5-trisphosphate production and other Ca^{2+} mobilizing agents. TRP ion channels influence calcium-depletion induced calcium influx processes in response to chemo-, mechano- and osmoregulatory events. ANKTM1, also designated TRPA1, plays a role in both nociceptor and hair cell transduction. Activation of ANKTM1 occurs by perception of noxious cold (less than 17 degrees Celsius) and pungent natural compounds, such as garlic, cinnamon oil and mustard oil. Inhibition of ANKTM1 impairs hair cell mechanotransduction. Blocking ANKTM1 may be a therapeutic target for treating cold hyperalgesia caused by inflammation and nerve damage.

REFERENCES

1. Corey, D.P., et al. 2004. TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells. *Nature* 432: 723-730.
2. Tominaga, M., et al. 2004. Thermosensation and pain. *J. Neurobiol.* 61: 3-12.
3. Bandell, M., et al. 2004. Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. *Neuron* 41: 849-857.
4. Obata, K., et al. 2005. TRPA1 induced in sensory neurons contributes to cold hyperalgesia after inflammation and nerve injury. *J. Clin. Invest.* 115: 2393-2401.
5. McKemy, D.D., et al. 2005. How cold is it? TRPM8 and TRPA1 in the molecular logic of cold sensation. *Mol. Pain* 1: 16.

CHROMOSOMAL LOCATION

Genetic locus: TRPA1 (human) mapping to 8q13.3.

PRODUCT

ANKTM1 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 μ l frozen stock containing 1.0×10^6 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 ANKTM1 siRNA (h): sc-44780 and ANKTM1 shRNA Plasmid (h): sc-44780-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

ANKTM1 shRNA (h) Lentiviral Particles is recommended for the inhibition of ANKTM1 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

ANKTM1 (C-5): sc-376495 is recommended as a control antibody for monitoring of ANKTM1 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-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ANKTM1 gene expression knockdown using RT-PCR Primer: ANKTM1 (h)-PR: sc-44780-PR (20 μ l, 579 bp). 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.