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ANKTM1 siRNA (h): sc-44780

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: 36.
6. Nagata, K., et al. 2005. Nociceptor and hair cell transducer properties of TRPA1, a channel for pain and hearing. *J. Neurosci.* 25: 4052-4061.

CHROMOSOMAL LOCATION

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

PRODUCT

ANKTM1 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 ANKTM1 shRNA Plasmid (h): sc-44780-SH and ANKTM1 shRNA (h) Lentiviral Particles: sc-44780-V as alternate gene silencing products.

For independent verification of ANKTM1 (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-44780A, sc-44780B and sc-44780C.

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

ANKTM1 siRNA (h) is recommended for the inhibition of ANKTM1 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

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 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 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.

RESEARCH USE

For research use only, not for use in diagnostic procedures.