

TRPV5 siRNA (h): sc-42676

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

CHROMOSOMAL LOCATION

Genetic locus: TRPV5 (human) mapping to 7q34.

PRODUCT

TRPV5 siRNA (h) is a pool of 2 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TRPV5 shRNA Plasmid (h): sc-42676-SH and TRPV5 shRNA (h) Lentiviral Particles: sc-42676-V as alternate gene silencing products.

For independent verification of TRPV5 (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-42676A and sc-42676B.

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 µl of the RNase-free water provided. Resuspension of the siRNA duplex in 330 µl of RNase-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

TRPV5 siRNA (h) is recommended for the inhibition of TRPV5 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 µM in 66 µl. 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

TRPV5 (B-6): sc-398853 is recommended as a control antibody for monitoring of TRPV5 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 TRPV5 gene expression knockdown using RT-PCR Primer: TRPV5 (h)-PR: sc-42676-PR (20 µl, 470 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Vachel, L., et al. 2015. The low PLC-δ1 expression in cystic fibrosis bronchial epithelial cells induces upregulation of TRPV6 channel activity. *Cell Calcium* 57: 38-48.

RESEARCH USE

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

PROTOCOLS

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