

ANO1 siRNA (h): sc-76686

BACKGROUND

ANO1 (anoctamin 1), also known as DOG1, ORAOV2, TAOS2 or TMEM16A, is a 986 amino acid multi-pass membrane protein that localizes to both the cell membrane and the cytoplasm and belongs to the anoctamin family. Expressed in a variety of tissues with highest expression in liver, gastrointestinal muscle and skeletal muscle, ANO1 functions as a calcium-activated chloride channel that is required for normal tracheal development. Human ANO1 shares 90% sequence identity with its mouse counterpart, suggesting a conserved role between species. ANO1 is present in breast, pancreatic, gastric, and uterine cancers, as well as in neck, ovarian and parathyroid tumors, suggesting a role for ANO1 in carcinogenesis. Three isoforms of ANO1 exist due to alternative splicing events.

REFERENCES

1. Katoh, M. and Katoh, M. 2003. FLJ10261 gene, located within the CCND1-EMS1 locus on human chromosome 11q13, encodes the eight-transmembrane protein homologous to C12orf3, C11orf25 and FLJ34272 gene products. *Int. J. Oncol.* 22: 1375-1381.
2. Katoh, M. and Katoh, M. 2004. Identification and characterization of TMEM16E and TMEM16F genes in silico. *Int. J. Oncol.* 24: 1345-1349.
3. Huang, X., et al. 2006. Comprehensive genome and transcriptome analysis of the 11q13 amplicon in human oral cancer and synteny to the 7F5 amplicon in murine oral carcinoma. *Genes Chromosomes Cancer* 45: 1058-1069.

CHROMOSOMAL LOCATION

Genetic locus: ANO1 (human) mapping to 11q13.3.

PRODUCT

ANO1 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 ANO1 shRNA Plasmid (h): sc-76686-SH and ANO1 shRNA (h) Lentiviral Particles: sc-76686-V as alternate gene silencing products.

For independent verification of ANO1 (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-76686A, sc-76686B and sc-76686C.

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

ANO1 siRNA (h) is recommended for the inhibition of ANO1 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

ANO1 (C-5): sc-377115 is recommended as a control antibody for monitoring of ANO1 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 ANO1 gene expression knockdown using RT-PCR Primer: ANO1 (h)-PR: sc-76686-PR (20 μ l, 523 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Matsuba, S., et al. 2014. Downregulation of Ca²⁺-activated Cl⁻ channel TMEM16A by the inhibition of histone deacetylase in TMEM16A-expressing cancer cells. *J. Pharmacol. Exp. Ther.* 351: 510-518.
2. Liu, J., et al. 2014. Transmembrane protein with unknown function 16A overexpression promotes glioma formation through the nuclear factor- κ B signaling pathway. *Mol. Med. Rep.* 9: 1068-1074.
3. Fujimoto, M., et al. 2017. Transcriptional repression of HER2 by ANO1 Cl⁻ channel inhibition in human breast cancer cells with resistance to trastuzumab. *Biochem. Biophys. Res. Commun.* 482: 188-194.
4. Fujimoto, M., et al. 2018. Transcriptional repression of human epidermal growth factor receptor 2 by CLC-3 Cl⁻/H⁺ transporter inhibition in human breast cancer cells. *Cancer Sci.* 109: 2781-2791.
5. Park, Y.R., et al. 2019. Down-regulation of miR-9 promotes epithelial mesenchymal transition via regulating anoctamin-1 (ANO1) in CRC cells. *Cancer Genet.* 231-232: 22-31.

RESEARCH USE

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