

CLC-2 siRNA (h): sc-42379

BACKGROUND

The family of voltage-dependent chloride channels (CLCs) regulate cellular trafficking of chloride ions, a critical component of all living cells. CLCs regulate excitability in muscle and nerve cells, aid in organic solute transport and maintain cellular volume. The genes encoding human CLC-1 through CLC-7 map to chromosomes 7q32, 3q28, 4q32, Xp22.3, Xp11.23, 1p36 and 16p13, respectively. CLC1 is highly expressed in skeletal muscle. Mutations in the gene encoding CLC1 lead to myotonia, an inheritable disorder characterized by muscle stiffness and renal salt wasting. CLC2 is highly expressed in the epithelia of several organs including lung, which suggests CLC2 may be a possible therapeutic target for cystic fibrosis. CLC3 expression is particularly abundant in neuronal tissue, while CLC4 expression is evident in skeletal and cardiac muscle as well as brain. Mutations in the gene encoding CLC5 lead to Dent's disease, a renal disorder characterized by proteinuria and hypercalciuria. CLC6 and CLC7 are broadly expressed in several tissues including testis, kidney, brain and muscle.

REFERENCES

1. Koch, M.C., et al. 1992. The skeletal muscle chloride channel in dominant and recessive human myotonia. *Science* 257: 797-800.
2. Pook, M.A., et al. 1993. Dent's disease, a renal Fanconi syndrome with nephrocalcinosis and kidney stones, is associated with a microdeletion involving DXS255 and maps to Xp11.22. *Hum. Mol. Genet.* 2: 2129-2134.
3. van Slegtenhorst, M.A., et al. 1994. A gene from the Xp22.3 region shares homology with voltage-gated chloride channels. *Hum. Mol. Genet.* 3: 547-552.

CHROMOSOMAL LOCATION

Genetic locus: CLCN2 (human) mapping to 3q27.1.

PRODUCT

CLC-2 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 CLC-2 shRNA Plasmid (h): sc-42379-SH and CLC-2 shRNA (h) Lentiviral Particles: sc-42379-V as alternate gene silencing products.

For independent verification of CLC-2 (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-42379A, sc-42379B and sc-42379C.

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

CLC-2 siRNA (h) is recommended for the inhibition of CLC-2 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

CLC-2 (D-6): sc-377284 is recommended as a control antibody for monitoring of CLC-2 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 CLC-2 gene expression knockdown using RT-PCR Primer: CLC-2 (h)-PR: sc-42379-PR (20 μ l, 428 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Nighot, P.K., et al. 2012. Chloride channel CLC-2 modulates tight junction barrier function via intracellular trafficking of occludin. *Am. J. Physiol., Cell Physiol.* 302: C178-C187.

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.