

CLC-2 siRNA (r): sc-61868

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, Xp11.22, 1p36 and 16p13, respectively. CLC-1 is highly expressed in skeletal muscle. Mutations in the gene encoding CLC-1 lead to myotonia, an inheritable disorder characterized by muscle stiffness and renal salt wasting. CLC-2 is highly expressed in the epithelia of several organs including lung, which suggests CLC-2 may be a possible therapeutic target for cystic fibrosis. CLC-3 expression is particularly abundant in neuronal tissue, while CLC-4 expression is evident in skeletal and cardiac muscle as well as brain. Mutations in the gene encoding CLC-5 lead to Dent's disease, a renal disorder characterized by proteinuria and hypercalciuria. CLC-6 and CLC-7 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.
4. Cid, L.P., et al. 1995. Cloning of a putative human voltage-gated chloride channel (CLC-2) cDNA widely expressed in human tissues. *Hum. Mol. Genet.* 4: 407-413.
5. Borsani, G., et al. 1995. Characterization of a human and murine gene (CLCN3) sharing similarities to voltage-gated chloride channels and to a yeast integral membrane protein. *Genomics* 27: 131-141.

CHROMOSOMAL LOCATION

Genetic locus: Clcn2 (rat) mapping to 11q23.

PRODUCT

CLC-2 siRNA (r) 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 (r): sc-61868-SH and CLC-2 shRNA (r) Lentiviral Particles: sc-61868-V as alternate gene silencing products.

For independent verification of CLC-2 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-61868A, sc-61868B and sc-61868C.

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

CLC-2 siRNA (r) is recommended for the inhibition of CLC-2 expression in rat 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 (r)-PR: sc-61868-PR (20 μ l, 578 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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