

CLCA2 siRNA (h): sc-60394

BACKGROUND

The calcium-activated chloride channel (CLCA) protein family, which includes the human homologs CLCA1 and CLCA2, display distinct tissue distribution patterns. CLCA1 is expressed as a precursor protein that is processed into two cell surface associated subunits and a group of proteins. CLCA1 is upregulated by interleukin-9 and regulates the expression of mucins. CLCA1 may provide a therapeutic target to control mucus overproduction in airway disease patients with cystic fibrosis. CLCA2 expression is downregulated in breast cancer and therefore, is thought to act as a tumor suppressor in normal cells. CLCA3 is a structurally divergent member of the CLCA family that does not function as a channel protein. CLCA4 is a CLCA member that is expressed in human rectal mucosa, CLCA5 shows strong expression in eye and spleen, and CLCA6 is primarily expressed in intestine and stomach.

REFERENCES

1. Gandhi, R., et al. 1998. Molecular and functional characterization of a calcium-sensitive chloride channel from mouse lung. *J. Biol. Chem.* 273: 32096-32101.
2. Gruber, A.D., et al. 1998. Genomic cloning, molecular characterization, and functional analysis of human CLCA1, the first human member of the family of Ca²⁺-activated Cl⁻ channel proteins. *Genomics* 54: 200-214.
3. Gruber, A.D., et al. 1999. Molecular cloning and transmembrane structure of hCLCA2 from human lung, trachea, and mammary gland. *Am. J. Physiol.* 276: 1261-1270.
4. Hauber, H.P., et al. 2003. Increased expression of interleukin-9, interleukin-9 receptor, and the calcium-activated chloride channel hCLCA1 in the upper airways of patients with cystic fibrosis. *Laryngoscope* 113: 1037-1042.
5. Beckley, J.R., et al. 2004. Re-expression of detachment-inducible chloride channel mCLCA5 suppresses growth of metastatic breast cancer cells. *J. Biol. Chem.* 279: 41634-41641.
6. Hauber, H.P., et al. 2004. Expression of hCLCA1 in cystic fibrosis lungs is associated with mucus overproduction. *Eur. Respir. J.* 23: 846-850.
7. Li, X., et al. 2004. CLCA2 tumour suppressor gene in 1p31 is epigenetically regulated in breast cancer. *Oncogene* 23: 1474-1480.

CHROMOSOMAL LOCATION

Genetic locus: CLCA2 (human) mapping to 1p22.3.

PRODUCT

CLCA2 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 CLCA2 shRNA Plasmid (h): sc-60394-SH and CLCA2 shRNA (h) Lentiviral Particles: sc-60394-V as alternate gene silencing products.

For independent verification of CLCA2 (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-60394A, sc-60394B and sc-60394C.

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

CLCA2 siRNA (h) is recommended for the inhibition of CLCA2 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

CLCA2 (1D5): sc-517015 is recommended as a control antibody for monitoring of CLCA2 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 CLCA2 gene expression knockdown using RT-PCR Primer: CLCA2 (h)-PR: sc-60394-PR (20 μ l). 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.

PROTOCOLS

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