

KCC1 siRNA (h): sc-42604

BACKGROUND

The four isoforms of potassium/chloride co-transport channels (KCC) belong to a superfamily of cation-chloride co-transporters involved in cell volume maintenance. Nitric oxide (NO) donors activate KCCs, while inhibitors of the cGMP pathway prevent NO donor activation. The ubiquitously expressed KCC1 contains 12 transmembrane domains with both cytoplasmic N and C terminal domains. KCC2 expression is limited to neuronal tissues by a restrictive element similar to the neuronal-restrictive silencing factor. In neurons, KCC2 expression is correlated with an inhibitory response to GABA, while the absence of KCC2 is necessary for an unusual excitatory response to GABA. Alterations of KCC2 expression in the inferior colliculus of rat brain may be related to seizure susceptibility. Conversely, KCC3 is not suspected to play a major role in epilepsy. The two splice variants of KCC3, KCC3 α and KCC3 β , are predominantly expressed in brain and kidney, respectively, while KCC4 is expressed in muscle, brain, lung, heart and kidney.

REFERENCES

1. Mount, D.B., et al. 1999. Cloning and characterization of KCC3 and KCC4, new members of the cation-chloride cotransporter gene family. *J. Biol. Chem.* 274: 16355-16362.
2. Lauf, P.K., et al. 2000. K-Cl cotransport: properties and molecular mechanism. *Cell. Physiol. Biochem.* 10: 341-354.
3. Di Fulvio, M., et al. 2001. Protein kinase G regulates potassium chloride cotransporter-3 expression in primary cultures of rat vascular smooth muscle cells. *J. Biol. Chem.* 276: 21046-21052.
4. Lauf, P.K., et al. 2001. K-Cl cotransport: immunohistochemical and ion flux studies in human embryonic kidney (HEK293) cells transfected with full-length and C-terminal-domain-truncated KCC1 cDNAs. *Cell. Physiol. Biochem.* 11: 143-160.
5. Karadsheh, M.F., et al. 2001. Neuronal restrictive silencing element is found in the KCC2 gene: molecular basis for KCC2-specific expression in neurons. *J. Neurophysiol.* 85: 995-997.
6. Kanaka, C., et al. 2001. The differential expression patterns of messenger RNAs encoding K-Cl cotransporters (KCC1,2) and Na-K-Cl cotransporter (NKCC1) in the rat nervous system. *Neuroscience* 104: 933-946.

CHROMOSOMAL LOCATION

Genetic locus: SLC12A4 (human) mapping to 16q22.1.

PRODUCT

KCC1 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 KCC1 shRNA Plasmid (h): sc-42604-SH and KCC1 shRNA (h) Lentiviral Particles: sc-42604-V as alternate gene silencing products.

For independent verification of KCC1 (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-42604A, sc-42604B and sc-42604C.

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

KCC1 siRNA (h) is recommended for the inhibition of KCC1 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

KCC1 (FT-94): sc-134370 is recommended as a control antibody for monitoring of KCC1 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 MarkerTM 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 KCC1 gene expression knockdown using RT-PCR Primer: KCC1 (h)-PR: sc-42604-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.