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SANTA CRUZ BIOTECHNOLOGY, INC.

CHT1 siRNA (m): sc-44595

BACKGROUND

Under physiological conditions, Na^+-Cl^--dependent hemicholinium-3 (HC-3)-sensitive, high-affinity choline uptake limits the rate of acetylcholine synthesis in cholinergic neurons. Hemicholinium-3 sensitive high-affinity choline transporter (CHT1) carries out this uptake. Regions of the nervous system that are rich with cholinergic cell bodies such as the spinal cord, brainstem, mid-brain, and striatum express CHT at high levels, whereas tissues lacking cholinergic cells, such as the cerebellum and kidney, show no CHT1 expression. CHT1 localizes to a subpopulation of small vesicles, which also contain vesicular acetylcholine transporter and acetylcholine, within the cholinergic presynaptic terminals. In response to neuronal activity, these particular vesicles translocate to the plasma membrane to re-uptake choline, a process that, due to the other contents of the vesicle, may be coupled with the rate of ACh release.

REFERENCES

1. Okuda, T., et al. 2000. Functional characterization of the human high-affinity choline transporter. FEBS Lett. 484: 92-97.
2. Apparsundaram, S., et al. 2000. Molecular cloning of a human, hemicholinium-3-sensitive choline transporter. Biochem. Biophys. Res. Commun. 276: 862-867.
3. Apparsundaram, S., et al. 2001. Molecular cloning and characterization of a murine hemicholinium-3-sensitive choline transporter. Biochem. Soc. Trans. 29: 711-716.
4. Haberberger, R.V., et al. 2002. Expression of the high-affinity choline transporter, CHT1, in the neuronal and non-neuronal cholinergic system of human and rat skin. J. Invest. Dermatol. 119: 943-948.
5. Ferguson, S.M., et al. 2003. Vesicular localization and activity-dependent trafficking of presynaptic choline transporters. J. Neurosci. 23: 9697-9709.
6. Ribeiro, F.M., et al. 2003. The hemicholinium-3 sensitive high affinity choline transporter is internalized by clathrin-mediated endocytosis and is present in endosomes and synaptic vesicles. J. Neurochem. 87: 136-146.

CHROMOSOMAL LOCATION

Genetic locus: Slc5a7 (mouse) mapping to 17 C.

PRODUCT

CHT1 siRNA (m) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CHT1 shRNA Plasmid (m): sc-44595-SH and CHT1 shRNA (m) Lentiviral Particles: sc-44595-V as alternate gene silencing products.

For independent verification of CHT1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44595A, sc-44595B and sc-44595C.

RESEARCH USE

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

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

CHT1 siRNA (m) is recommended for the inhibition of CHT1 expression in mouse 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 µM in 66 µl. 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

CHT1 (62-2ES): sc-33713 is recommended as a control antibody for monitoring of CHT1 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 CHT1 gene expression knockdown using RT-PCR Primer: CHT1 (m)-PR: sc-44595-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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