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SANTA CRUZ BIOTECHNOLOGY, INC.

choactase siRNA (m): sc-41920

BACKGROUND

Choline acetyltransferase (also designated choactase, choline O-acetyltransferase) synthesizes acetylcholine in cholinergic neurons. Multiple choactase mRNAs with different 5'-noncoding regions are expressed as R-, N1, N2-, S- and M-types. N1-, N2- and R-type mRNAs produce a single short enzyme, while M-type mRNA produces both long and short enzymes. The long enzyme is targeted to the nuclei of cells, whereas the short protein is found in cytoplasm. A novel NFκB binding site is located within the nerve growth factor-responsive enhancer element that is recognized by the NFκB protein p49, but not p65 or p50. Decreased choactase expression and increased NFκB activity are associated with aging and Alzheimer's disease, indicating that p49 is a negative regulator of choactase expression and suggesting a possible mechanism for aging-associated declines in cholinergic function. Phosphorylation of choactase has been shown to enhance choactase catalytic activity. Specifically, Serine 440 is found to be the phosphorylation site in a recombinant human short choactase by protein kinase C and is involved in regulation of the enzyme catalytic activity and binding to subcellular membranes.

REFERENCES

1. Oda, Y., et al. 1992. A complementary DNA for human choline acetyltransferase induces two forms of enzyme with different molecular weights in cultured cells. Brain Res. Mol. Brain Res. 16: 287-294.
2. Misawa, H., et al. 1997. Human choline acetyltransferase mRNAs with different 5'-region produce a 69 kDa major translation product. Brain Res. Mol. Brain Res. 44: 323-333.
3. Resendes, M.C., et al. 1999. Nuclear localization of the 82 kDa form of human choline acetyltransferase. J. Biol. Chem. 274: 19417-197421.
4. Dobransky, T., et al. 2000. Expression, purification and characterization of recombinant human cholineacetyltransferase: phosphorylation of the enzyme regulates catalytic activity. Biochem. J. 349: 141-151.
5. Toliver-Kinsky, T., et al. 2000. Nuclear factor κB/p49 is a negative regulatory factor in nerve growth factor-induced choline acetyltransferase promoter activity in PC12 cells. J. Neurochem. 75: 2241-2251.
6. Dobransky, T., et al. 2001. Functional characterization of phosphorylation of 69 kDa human choline acetyltransferase at serine 440 by protein kinase C. J. Biol. Chem. 276: 22244-22250.

CHROMOSOMAL LOCATION

Genetic locus: Chat (mouse) mapping to 14 B.

PRODUCT

choactase 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 choactase shRNA Plasmid (m): sc-41920-SH and choactase shRNA (m) Lentiviral Particles: sc-41920-V as alternate gene silencing products.

For independent verification of choactase (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-41920A, sc-41920B and sc-41920C.

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

choactase siRNA (m) is recommended for the inhibition of choactase 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

choactase (E-7): sc-55557 is recommended as a control antibody for monitoring of choactase 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 choactase gene expression knockdown using RT-PCR Primer: choactase (m)-PR: sc-41920-PR (20 µl). 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.