SANTA CRUZ BIOTECHNOLOGY, INC.

AChE siRNA (m): sc-29629

BACKGROUND

Acetylcholinesterase (AChE) hydrolyzes acetylcholine at synaptic junctions. Alternative mRNA splicing gives rise to three forms of AChE. The T form, also known as the asymmetric form, is soluble and is present in synapses. The H form is also known as the globular form and is present on the outer surfaces of cell membranes. The R form is not known to be a functional species. AChE globular form subunits are GPI-anchored to cell membranes and asymmetric subunits are anchored to basal lamina components by a collagen tail. The catalytic subunits of AChE are oligomers composed of disulfide-linked homodimers. The loss of AChE from cholinergic and noncholinergic neurons in the brain is seen in patients with Alzheimer's disease. However, AChE activity is increased around amyloid plaques, which may be due to a disturbance in calcium homeostasis involving the opening of L-type voltage-dependent calcium channels.

REFERENCES

1. Roberts, W.L., et al. 1991. Bovine brain acetylcholinesterase primary sequence involved in intersubunit disulfide linkages. J. Biol. Chem. 266: 7481-7487.
2. Duval, N., et al. 1992. H and T subunits of acetylcholinesterase from Torpedo, expressed in COS cells, generate all types of globular forms. J. Cell Biol. 118: 641-653.
3. Legay, C., et al. 1993. Cloning and expression of a rat acetylcholinesterase subunit: generation of multiple molecular forms and complementarity with a Torpedo collagenic subunit. J. Neurochem. 60: 337-346.
4. Legay, C., et al. 1993. Expression of a cDNA encoding the glycolipid-anchored form of rat acetylcholinesterase. FEBS Lett. 315: 163-166.
5. Michel, R.N., et al. 1994. Neural regulation of acetylcholinesterase mRNAs at mammalian neuromuscular synapses. J. Cell Biol. 127: 1061-1069.
6. Sberna, G., et al. 1997. The amyloid β-protein of Alzheimer's disease increases acetylcholinesterase expression by increasing intracellular calcium in embryonal carcinoma P19 cells. J. Neurochem. 69: 1177-1184.

CHROMOSOMAL LOCATION

Genetic locus: Ache (mouse) mapping to 5 G2.

PRODUCT

AChE 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 AChE shRNA Plasmid (m): sc-29629-SH and AChE shRNA (m) Lentiviral Particles: sc-29629-V as alternate gene silencing products.

For independent verification of AChE (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-29629A, sc-29629B and sc-29629C.

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

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

AChE (A-11): sc-373901 is recommended as a control antibody for monitoring of AChE 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 AChE gene expression knockdown using RT-PCR Primer: AChE (m)-PR: sc-29629-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.