

AChR α 4 siRNA (h): sc-42528

BACKGROUND

Members of the ligand-gated ion channel receptor family are characterized by their fast transmitting response to neurotransmitters. Two important members of this family are the nicotinic acetylcholine and glutamate receptors, both of which are composed of five homologous subunits forming a transmembrane aqueous pore. These transmembrane receptors change conformation in response to their cognate neurotransmitter. Nicotinic acetylcholine receptors are found at the postsynaptic membrane of the neuromuscular junction and bind acetylcholine molecules, allowing ions to move through the pore. Glutamate receptors are found in the postsynaptic membrane of cells in the central nervous system. The activity that is generated at the synapse by the binding of acetylcholine is terminated by acetylcholinesterase, an enzyme that rapidly hydrolyzes acetylcholine. AChR α 4, also known as EBN, BFNC, EBN1, NACHR, NACRA4, NACHRA4 or CHRNA4, is a 627 amino acid multi-pass membrane protein associated with nocturnal frontal lobe epilepsy type 1 (ENFL1), an autosomal dominant epilepsy characterized by nocturnal seizures with hyperkinetic automatisms and poorly organized stereotyped movements.

REFERENCES

1. Alkondon, M., et al. 1988. Acetylcholinesterase reactivators modify the functional properties of the nicotinic acetylcholine receptor ion channel. *J. Pharmacol. Exp. Ther.* 245: 543-556.
2. Betz, H. 1990. Ligand-gated ion channels in the brain: the amino acid receptor superfamily. *Neuron* 5: 383-392.

CHROMOSOMAL LOCATION

Genetic locus: CHRNA4 (human) mapping to 20q13.33.

PRODUCT

AChR α 4 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 AChR α 4 shRNA Plasmid (h): sc-42528-SH and AChR α 4 shRNA (h) Lentiviral Particles: sc-42528-V as alternate gene silencing products.

For independent verification of AChR α 4 (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-42528A, sc-42528B and sc-42528C.

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

AChR α 4 siRNA (h) is recommended for the inhibition of AChR α 4 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

AChR α 4 (A-6): sc-74519 is recommended as a control antibody for monitoring of AChR α 4 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 AChR α 4 gene expression knockdown using RT-PCR Primer: AChR α 4 (h)-PR: sc-42528-PR (20 μ l, 513 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Sun, X., et al. 2009. Rosiglitazone inhibits α 4 nicotinic acetylcholine receptor expression in human lung carcinoma cells through peroxisome proliferator-activated receptor γ -independent signals. *Mol. Cancer Ther.* 8: 110-118.

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.