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GABA_A R_γ2 siRNA (h): sc-42449

BACKGROUND

GAD-65 and GAD-67, glutamate decarboxylases, function to catalyze the production of GABA (γ -aminobutyric acid). In the central nervous system, GABA functions as the main inhibitory transmitter by increasing a Cl⁻ (chloride) conductance that inhibits neuronal firing. GABA has been shown to activate both ionotropic (GABA_A) and metabotropic (GABA_B) receptors, as well as a third class of receptors called GABA_C. The γ subunit of GABA_A receptors are important for benzodiazepine binding and modulation of GABA-mediated Cl⁻ current. GABA_A R_γ2 is a 467 amino acid multi-pass membrane protein localized to the postsynaptic cell membrane. Present as a pentamer with other GABA_A receptor chains (α , β , γ , δ and ρ), the GABA_A ligand-gated Cl⁻ channels selectively complex with D5DR to enable mutual inhibitory functional interactions between the two receptor systems. Defects in the gene encoding GABA_A R_γ2 have been found to be the cause of childhood absence epilepsy type 2, familial febrile convulsions type 8, generalized epilepsy with febrile seizures plus type 3 and severe myoclonic epilepsy in infancy.

REFERENCES

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2. Wang, H., et al. 1999. GABA_A-receptor-associated protein links GABA_A receptors and the cyto-skeleton. *Nature* 397: 69-72.
3. Kucken, A.M., et al. 2000. Identification of benzodiazepine binding site residues in the γ 2 subunit of the GABA_A receptor. *Mol. Pharmacol.* 57: 932-939.
4. Liu, F., et al. 2000. Direct protein-protein coupling enables cross-talk between dopamine D5 and GABA_A receptors. *Nature* 403: 274-280.
5. Baulac, S., et al. 2001. First genetic evidence of GABA_A receptor dysfunction in epilepsy: a mutation in the γ 2 subunit gene. *Nat. Genet.* 28: 46-48.
6. Kananura, C., et al. 2002. A splice-site mutation in GABRG2 associated with childhood absence epilepsy and febrile convulsions. *Arch. Neurol.* 59: 1137-1141.
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CHROMOSOMAL LOCATION

Genetic locus: GABRG2 (human) mapping to 5q34.

PRODUCT

GABA_A R_γ2 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 GABA_A R_γ2 shRNA Plasmid (h): sc-42449-SH and GABA_A R_γ2 shRNA (h) Lentiviral Particles: sc-42449-V as alternate gene silencing products.

For independent verification of GABA_A R_γ2 (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-42449A, sc-42449B and sc-42449C.

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

GABA_A R_γ2 siRNA (h) is recommended for the inhibition of GABA_A R_γ2 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor GABA_A R_γ2 gene expression knockdown using RT-PCR Primer: GABA_A R_γ2 (h)-PR: sc-42449-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.