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GABA_A R α 5 siRNA (r): sc-270130

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⁻ 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. Both GABA_A and GABA_C are ligand-gated ion channels, however, they are structurally and functionally distinct. Members of the GABA_A receptor family include GABA_A R α 1-6, GABA_A R β 1-3, GABA_A R γ 1-3, GABA_A R δ , GABA_A R ϵ , GABA_A R ρ 1 and GABA_A R ρ 2. The GABA_B family is composed of GABA_B R1 α and GABA_B R1 β . GABA transporters have also been identified and include GABA T-1, GABA T-2 and GABA T-3 (also designated GAT-1, -2, and -3). The GABA transporters function to terminate GABA action.

REFERENCES

- Nelson, H., et al. 1990. Cloning of the human brain GABA transporter. *FEBS Lett.* 269: 181-184.
- Cherubini, E., et al. 1991. GABA: an excitatory transmitter in early postnatal life. *Trends Neurosci.* 14: 515-519.
- Borden, L.A., et al. 1992. Molecular heterogeneity of the γ -aminobutyric acid (GABA) transport system. Cloning of two novel high affinity GABA transporters from rat brain. *J. Biol. Chem.* 267: 21098-21104.
- Dirx, R., Jr., et al. 1995. Targeting of the 67-kDa isoform of glutamic acid decarboxylase to intracellular organelles is mediated by its interaction with the NH₂-terminal region of the 65-kDa isoform of glutamic acid decarboxylase. *J. Biol. Chem.* 270: 2241-2246.

CHROMOSOMAL LOCATION

Genetic locus: Gabra5 (rat) mapping to 1q22.

PRODUCT

GABA_A R α 5 siRNA (r) 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 α 5 shRNA Plasmid (r): sc-270130-SH and GABA_A R α 5 shRNA (r) Lentiviral Particles: sc-270130-V as alternate gene silencing products.

For independent verification of GABA_A R α 5 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270130A, sc-270130B and sc-270130C.

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 α 5 siRNA (r) is recommended for the inhibition of GABA_A R α 5 expression in rat 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

GABA_A R α 5 (E-8): sc-376282 is recommended as a control antibody for monitoring of GABA_A R α 5 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 GABA_A R α 5 gene expression knockdown using RT-PCR Primer: GABA_A R α 5 (r)-PR: sc-270130-PR (20 μ l, 510 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- De la Luz-Cuellar, Y.E., et al. 2019. Blockade of spinal α ₅-GABA_A receptors differentially reduces reserpine-induced fibromyalgia-type pain in female rats. *Eur. J. Pharmacol.* 858: 172443.

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.