SANTA CRUZ BIOTECHNOLOGY, INC.

$GABA_A$ Rδ siRNA (m): sc-42444

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 $GABA_A$ Rα1-6, $GABA_A$ Rβ1-3, $GABA_A$ Rγ1-3, $GABA_A$ Rδ, $GABA_A$ Rε, $GABA_A$ Rρ1 and $GABA_A$ Rρ2. The $GABA_B$ family is composed of $GABA_B$ R1α and $GABA_B$ R1β. 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

1. Nelson, H., et al. 1990. Cloning of the human brain GABA transporter. FEBS Lett. 269: 181-184.
2. Cherubini, E., et al. 1991. GABA: an excitatory transmitter in early postnatal life. Trends Neurosci. 14: 515-519.
3. 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.
4. Dirkx, 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 NH_2-terminal region of the 65-kDa isoform of glutamic acid decarboxylase. J. Biol. Chem. 270: 2241-2246.
5. Lukasiewicz, P.D. 1996. $GABA_C$ receptors in the vertebrate retina. Mol. Neurobiol. 12: 181-194.
6. Kaupmann, K., et al. 1997. Expression cloning of $GABA_B$ receptors uncovers similarity to metabotropic glutamate receptors. Nature 386: 239-246.
7. Korpi, E.R., et al. 1997. $GABA_A$-receptor subtypes: clinical efficiency and selectivity of benzodiazepine site ligands. Ann. Med. 29: 275-282.

CHROMOSOMAL LOCATION

Genetic locus: Gabrd (mouse) mapping to 4 E2.

PRODUCT

$GABA_A$ Rδ 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 $GABA_A$ Rδ shRNA Plasmid (m): sc-42444-SH and $GABA_A$ Rδ shRNA (m) Lentiviral Particles: sc-42444-V as alternate gene silencing products.

For independent verification of $GABA_A$ Rδ (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-42444A, sc-42444B and sc-42444C.

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

$GABA_A$ Rδ siRNA (m) is recommended for the inhibition of $GABA_A$ Rδ 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

$GABA_A$ Rδ (H-4): sc-271231 is recommended as a control antibody for monitoring of $GABA_A$ Rδ 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 $GABA_A$ Rδ gene expression knockdown using RT-PCR Primer: $GABA_A$ Rδ (m)-PR: sc-42444-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.