

GABA T-3 siRNA (h): sc-41962

BACKGROUND

As glutamate decarboxylases, GAD-65 and GAD-67 function to catalyze the production of GABA (γ -aminobutyric acid). In the central nervous system, GABA functions as the main inhibitory transmitter by increasing the chlorine 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. 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). GABA T-3 is a 632 amino acid membrane protein that is expressed in brain, specifically in glial cells. The GABA transporters function to terminate GABA action by actively pumping GABA back into presynaptic terminals.

REFERENCES

1. Takayama, C. and Inoue, Y. 2005. Developmental expression of GABA transporter-1 and 3 during formation of the GABAergic synapses in the mouse cerebellar cortex. *Brain Res. Dev. Brain Res.* 158: 41-49.
2. Pow, D.V., et al. 2005. Differential expression of the GABA transporters GAT-1 and GAT-3 in brains of rats, cats, monkeys and humans. *Cell Tissue Res.* 320: 379-392.
3. Melone, M., et al. 2005. Neuronal localization of the GABA transporter GAT-3 in human cerebral cortex: a procedural artifact? *J. Chem. Neuroanat.* 30: 45-54.
4. Galvan, A., et al. 2005. GABAergic modulation of the activity of globus pallidus neurons in primates: *in vivo* analysis of the functions of GABA receptors and GABA transporters. *J. Neurophysiol.* 94: 990-1000.
5. Kinney, G.A. 2005. GAT-3 transporters regulate inhibition in the neocortex. *J. Neurophysiol.* 94: 4533-4537.
6. Birnbaum, A.D., et al. 2005. Cloning, immunolocalization, and functional expression of a GABA transporter from the retina of the skate. *Vis. Neurosci.* 22: 211-223.
7. Lee, T.S., et al. 2006. GAT-1 and GAT-3 expression are differently localized in the human epileptogenic hippocampus. *Acta Neuropathol.* 111: 351-363.

CHROMOSOMAL LOCATION

Genetic locus: SLC6A11 (human) mapping to 3p25.3.

PRODUCT

GABA T-3 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 T-3 shRNA Plasmid (h): sc-41962-SH and GABA T-3 shRNA (h) Lentiviral Particles: sc-41962-V as alternate gene silencing products.

For independent verification of GABA T-3 (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-41962A, sc-41962B and sc-41962C.

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 T-3 siRNA (h) is recommended for the inhibition of GABA T-3 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

GABA T-3 (G-6): sc-376001 is recommended as a control antibody for monitoring of GABA T-3 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 T-3 gene expression knockdown using RT-PCR Primer: GABA T-3 (h)-PR: sc-41962-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.