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GABARAP siRNA (m): sc-41957

BACKGROUND

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. In addition to GABA receptors, several proteins have been identified as regulators of GABA function, including GAD65, GAD67, GABA transporters and GABARAP (GABA_A receptor-associated protein). GABARAP associates with GABA_A Ry2 to link GABA_A receptors to the cytoskeleton. The GABARAP protein sequence is similar to light chain-3 of microtubule-associated proteins (MAPs), suggesting that it may be a type of MAP or a component of a MAP complex.

REFERENCES

1. Cherubini, E., et al. 1991. GABA: an excitatory transmitter in early post-natal life. *Trends Neurosci.* 14: 515-519.
2. 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. *Biol. Chem.* 270: 2241-2246.
3. Lukasiewicz, P.D. 1996. GABA_C receptors in the vertebrate retina. *Mol. Neurobiol.* 12: 181-194.
4. Borden, L.A. 1996. GABA transporter heterogeneity: pharmacology and cellular localization. *Neurochem. Int.* 29: 335-356.
5. Kaupmann, K., et al. 1998. Human γ -aminobutyric acid type B receptors are differentially expressed and regulate inwardly rectifying K⁺ channels. *Proc. Natl. Acad. Sci. USA* 95: 14991-14996.
6. Wang, H., et al. 1999. GABA_A-receptor-associated protein links GABA_A receptors and the cytoskeleton. *Nature* 397: 69-72.

CHROMOSOMAL LOCATION

Genetic locus: Gabarap (mouse) mapping to 11 B4.

PRODUCT

GABARAP 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GABARAP shRNA Plasmid (m): sc-41957-SH and GABARAP shRNA (m) Lentiviral Particles: sc-41957-V as alternate gene silencing products.

For independent verification of GABARAP (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-41957A, sc-41957B and sc-41957C.

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.

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

GABARAP siRNA (m) is recommended for the inhibition of GABARAP 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 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

GABARAP (E-8): sc-377300 is recommended as a control antibody for monitoring of GABARAP 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 GABARAP gene expression knockdown using RT-PCR Primer: GABARAP (m)-PR: sc-41957-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.