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GABA_B R1 siRNA (h): sc-42459

BACKGROUND

In the central nervous system (CNS), γ -aminobutyric acid (GABA) is the main inhibitory neurotransmitter that functions to regulate neuronal firing. GABA exerts its effects through two different kinds of receptors: ionotropic receptors (GABA_A R and GABA_C R), which produce fast inhibitory signals, and metabotropic receptors (GABA_B R), which produce slow inhibitory signals. The GABA_B R receptor is a heterodimer that consists of two multi-pass membrane proteins, designated GABA_B R1 and GABA_B R2, both of which belong to the G protein-coupled receptor family and are highly expressed in brain tissue. Together, GABA_B R1 and GABA_B R2 play a crucial role in the fine-tuning of inhibitory synaptic transmissions and are implicated in slow wave sleep, muscle relaxation, hippocampal long-term potentiation and antinociception events. Both GABA_B R1 and GABA_B R2 are regulated by G proteins that have a variety of functions, including activation of potassium channels, inhibition of adenylyl cyclase (A cyclase) activity and modulation of inositol phospholipid hydrolysis.

REFERENCES

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- Balasubramanian, S., et al. 2004. Hetero-oligomerization between GABA_A and GABA_B receptors regulates GABA_B receptor trafficking. *J. Biol. Chem.* 279: 18840-18850.
- Brock, C., et al. 2005. Assembly-dependent surface targeting of the heterodimeric GABA_B Receptor is controlled by COPI but not 14-3-3. *Mol. Biol. Cell* 16: 5572-5578.
- Osawa, Y., et al. 2006. Functional expression of the GABA_B receptor in human airway smooth muscle. *Am. J. Physiol. Lung Cell Mol. Physiol.* 291: L923-L931.
- Chang, W., et al. 2007. Complex formation with the Type B γ -aminobutyric acid receptor affects the expression and signal transduction of the extracellular calcium-sensing receptor. *Studies with HEK-293 cells and neurons.* *J. Biol. Chem.* 282: 25030-25040.

CHROMOSOMAL LOCATION

Genetic locus: GABBR1 (human) mapping to 6p22.1.

PRODUCT

GABA_B R1 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_B R1 shRNA Plasmid (h): sc-42459-SH and GABA_B R1 shRNA (h) Lentiviral Particles: sc-42459-V as alternate gene silencing products.

For independent verification of GABA_B R1 (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-42459A, sc-42459B and sc-42459C.

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_B R1 siRNA (h) is recommended for the inhibition of GABA_B R1 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_B R1 (D-2): sc-166408 is recommended as a control antibody for monitoring of GABA_B R1 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_B R1 gene expression knockdown using RT-PCR Primer: GABA_B R1 (h)-PR: sc-42459-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.