SANTA CRUZ BIOTECHNOLOGY, INC.

$GABA_B$ R2 siRNA (m): sc-42464

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

1. White, J.H., et al. 2000. The $GABA_B$ receptor interacts directly with the related transcription factors CREB2 and ATF_x. Proc. Natl. Acad. Sci. USA 97: 13967-13972.
2. 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.
3. 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.
4. 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.
5. 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: Gabbr2 (mouse) mapping to 4 B1.

PRODUCT

$GABA_B$ R2 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 $GABA_B$ R2 shRNA Plasmid (m): sc-42464-SH and $GABA_B$ R2 shRNA (m) Lentiviral Particles: sc-42464-V as alternate gene silencing products.

For independent verification of $GABA_B$ R2 (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-42464A, sc-42464B and sc-42464C.

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

$GABA_B$ R2 siRNA (m) is recommended for the inhibition of $GABA_B$ R2 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

$GABA_B$ R2 (H-10): sc-393270 is recommended as a control antibody for monitoring of $GABA_B$ R2 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 $GABA_B$ R2 gene expression knockdown using RT-PCR Primer: $GABA_B$ R2 (m)-PR: sc-42464-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.