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# GABA<sub>A</sub> R $\epsilon$ siRNA (h): sc-42445

## BACKGROUND

GAD-65 and GAD-67, glutamate decarboxylases, function to catalyze the production of GABA ( $\gamma$ -aminobutyric acid). In the central nervous system GABA functions as the main inhibitory transmitter by increasing a Cl<sup>-</sup> conductance that inhibits neuronal firing. GABA has been shown to activate both ionotropic (GABA<sub>A</sub>) and metabotropic (GABA<sub>B</sub>) receptors as well as a third class of receptors called GABA<sub>C</sub>. Both GABA<sub>A</sub> and GABA<sub>C</sub> are ligand-gated ion channels, however, they are structurally and functionally distinct. Members of the GABA<sub>A</sub> receptor family include GABA<sub>A</sub> R $\alpha$ 1-6, GABA<sub>A</sub> R $\beta$ 1-3, GABA<sub>A</sub> R $\gamma$ 1-3, GABA<sub>A</sub> R $\delta$ , GABA<sub>A</sub> R $\epsilon$ , GABA<sub>A</sub> R $\rho$ 1 and GABA<sub>A</sub> R $\rho$ 2. The GABA<sub>B</sub> family is composed of GABA<sub>B</sub> R1 $\alpha$  and GABA<sub>B</sub> R1 $\beta$ . 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  $\gamma$ -aminobutyric acid (GABA) transport system. Cloning of two novel high affinity GABA transporters from rat brain. *J. Biol. Chem.* 267: 21098-21104.
4. 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 NH<sub>2</sub>-terminal region of the 65-kDa isoform of glutamic acid decarboxylase. *J. Biol. Chem.* 270: 2241-2246.
5. Lukasiewicz, P.D. 1996. GABA<sub>C</sub> receptors in the vertebrate retina. *Mol. Neurobiol.* 12: 181-194.
6. Kaupmann, K., et al. 1997. Expression cloning of GABA<sub>B</sub> receptors uncovers similarity to metabotropic glutamate receptors. *Nature* 386: 239-246.

## CHROMOSOMAL LOCATION

Genetic locus: GABRE (human) mapping to Xq28.

## PRODUCT

GABA<sub>A</sub> R $\epsilon$  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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GABA<sub>A</sub> R $\epsilon$  shRNA Plasmid (h): sc-42445-SH and GABA<sub>A</sub> R $\epsilon$  shRNA (h) Lentiviral Particles: sc-42445-V as alternate gene silencing products.

For independent verification of GABA<sub>A</sub> R $\epsilon$  (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-42445A, sc-42445B and sc-42445C.

## PROTOCOLS

See our web site at [www.scbt.com](http://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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

GABA<sub>A</sub> R $\epsilon$  siRNA (h) is recommended for the inhibition of GABA<sub>A</sub> R $\epsilon$  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  $\mu$ M in 66  $\mu$ 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<sub>A</sub> R $\epsilon$  (E-12): sc-271668 is recommended as a control antibody for monitoring of GABA<sub>A</sub> R $\epsilon$  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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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<sub>A</sub> R $\epsilon$  gene expression knockdown using RT-PCR Primer: GABA<sub>A</sub> R $\epsilon$  (h)-PR: sc-42445-PR (20  $\mu$ 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.