SANTA CRUZ BIOTECHNOLOGY, INC.

VGAT siRNA (r): sc-270411

BACKGROUND

Synaptic transmission involves the controlled exocytosis of vesicles containing specific neurotransmitters. Usually, neurotransmitters are synthesized in the cytoplasm of the cell and must be transported into synaptic vesicles for release. The vesicular GABA transporter (VGAT) is responsible for loading γ-aminobutyric acid (GABA), an inhibitory neurotransmitter, from neuronal cytoplasm into synaptic vesicles and is expressed only in the nerve endings of inhibitory neurons that contain GABA and/or glycine. During neocortical development, VGAT expression barely precedes the maturation of inhibitory synaptogenesis, suggesting that it may contribute to the development of neocortical GABAergic circuitry. VGAT may also play a role in epileptogenesis and the recovery mechanisms that occur after a spontaneous seizure.

REFERENCES

1. Minelli, A., et al. 2003. Postnatal development of the vesicular GABA transporter in rat cerebral cortex. Neuroscience 117: 337-346.
2. Conti, F., et al. 2005. Heterogeneity of axon terminals expressing VGLUT1 in the cerebral neocortex. Arch. Ital. Biol. 143: 127-132.
3. End, K., et al. 2005. Receptors and sites of synthesis and storage of γ-aminobutyric acid in human pituitary glands and in growth hormone adenomas. Am. J. Clin. Pathol. 124: 550-558.
4. Gerstein, M., et al. 2005. Remodeling of H hypoxia and magnesium sulfate load: immunohistochemical study. Exp. Neurol. 196: 18-29.
5. Oh, W.J., et al. 2005. The mouse vesicular inhibitory amino acid transporter gene: expression during embryogenesis, analysis of its core promoter in neural stem cells and a reconsideration of its alternate splicing. Gene 351: 39-49.
6. Vemuganti, R. 2005. Decreased expression of vesicular GABA transporter, but not vesicular glutamate, acetylcholine and monoamine transporters in rat brain following focal ischemia. Neurochem. Int. 47: 136-142.
7. Zink, M. and Spanagel, R. 2005. Ethanol induces GAD67 and VGAT in slice cultures of newborn rat cerebral cortex. Neuroreport 16: 377-380.
8. Bogen, I.L., et al. 2006. Absence of Synapsin I and II is accompanied by decreases in vesicular transport of specific neurotransmitters. J. Neurochem. 96: 1458-1466.

CHROMOSOMAL LOCATION

Genetic locus: Slc32a1 (rat) mapping to 3q42.

PRODUCT

VGAT siRNA (r) is a pool of 2 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 VGAT shRNA Plasmid (r): sc-270411-SH and VGAT shRNA (r) Lentiviral Particles: sc-270411-V as alternate gene silencing products.

For independent verification of VGAT (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270411A and sc-270411B.

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

VGAT siRNA (r) is recommended for the inhibition of VGAT expression in rat 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

VGAT (F-2): sc-393373 is recommended as a control antibody for monitoring of VGAT 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 VGAT gene expression knockdown using RT-PCR Primer: VGAT (r)-PR: sc-270411-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.