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SANTA CRUZ BIOTECHNOLOGY, INC.

Synaptotagmin I/II shRNA (h) Lentiviral Particles: sc-44135-V

BACKGROUND

Synaptotagmins are a large gene family of synaptic vesicle type III integral membrane proteins that function as regulators of both exocytosis and endocytosis and are involved in neurotransmitter secretion from small secretory vesicles. Calcium binds to Synaptotagmin I which triggers neurotransmitter release at the synapse. Synaptotagmin II is phosphorylated by WNK1 in a process that regulates calcium-dependent interactions. Synaptotagmin III is involved in calcium-dependent exocytosis of secretory vesicles in endocrine cells and neurons. Synaptotagmin IV is expressed in neuronal tissues, and has the highest mRNA levels in the hippocampus. The proximity of the Synaptotagmin IV gene to markers of several psychiatric disorders suggest an involvement of synaptotagmin IV in human disease. Synaptotagmin V is a dense-core vesicle-specific protein that regulates a specific type of calcium-regulated secretion. Synaptotagmin VI interacts with adaptor protein-2 in a calcium-independent manner. Synaptotagmin VII is widely expressed in non-neuronal tissues.

REFERENCES

1. Hilbush, B.S. and Morgan, J.I. 1994. A third synaptotagmin gene, Syt3, in the mouse. Proc. Natl. Acad. Sci. USA. 91: 8195-8199.
2. Li, C., Ullrich, B., Zhang, J.Z., Anderson, R.G., Brose, N., and Sudhof, T.C. 1995. Ca2+-dependent and -independent activities of neural and non-neural synaptotagmins. Nature 375: 594-599.
3. Kishore, B.K., Wade, J.B., Schorr, K., Inoue, T., Mandon, B., and Knepper, M.A. 1998 Expression of synaptotagmin VIII in rat kidney. Am. J. Physiol. 275: F131-F142.
4. Xi, D., Chin, H., and Gainer, H. 1999. Analysis of synaptotagmin I-IV messenger RNA expression and developmental regulation in the rat hypothalamus and pituitary. Neuroscience 88: 425-435.

CHROMOSOMAL LOCATION

Genetic locus: SYT1 (human) mapping to 12q21.2, SYT2 (human) mapping to 1q32.1.

PRODUCT

Synaptotagmin I/II shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 4 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Synaptotagmin I/II siRNA (h): sc-44135 and Synaptotagmin I/II shRNA Plasmid (h): sc-44135-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

Synaptotagmin I/II shRNA (h) Lentiviral Particles is recommended for the inhibition of Synaptotagmin I and Synaptotagmin II expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

Synaptotagmin I/II (H-9): sc-393392 is recommended as a control antibody for monitoring of Synaptotagmin I/II 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Synaptotagmin I and Synaptotagmin II gene expression knockdown using RT-PCR Primer: Synaptotagmin I/II (h)-PR: sc-44135-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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