SANTA CRUZ BIOTECHNOLOGY, INC.

SYP siRNA (m): sc-36596

BACKGROUND

Synaptic vesicles participate in a cycle of fusion with the plasma membrane and reformation by endocytosis. Synaptic vesicle protein synaptophysin (SYP) is targeted to early endosomes in transfected fibroblasts and in neuroendocrine cells. SYP is an N-glycosylated intergral membrane protein found in neurons and endocrine cells that associates into hexamers to form a large conductance channel. SYP contains four transmembrane domains and may function as a gap junction-like channel. Membrane cholesterol specfically interacts with SYP to play a role in vesicle formation. Synaptobrevin (VAMP) also binds to SYP and the resultant complex is upregulated during neuronal development, but is absent in exocytosis fusion complex. Thus, the synaptophysin-synaptobrevin complex is not essential for exocytosis, but rather provides a pool of synaptobrevin for exocytosis. In addition, the tail domain of brain Myosin V also forms a stable complex with synaptobrevin II and SYP, and this complex is disassembled upon the depolarization-induced entry of Ca^{2+} into intact nerve endings.

REFERENCES

1. Cowan, D., et al. 1990. Torpedo synaptophysin: evolution of a synaptic vesicle protein. Brain Res. 509: 1-7.
2. Linstedt, A.D. and Kelly, R.B. 1991. Endocytosis of the synaptic vesicle protein, synaptophysin, requires the COOH-terminal tail. J. Physiol. 85: 90-96.
3. Calakos, N. and Scheller, R.H. 1994. Vesicle-associated membrane protein and synaptophysin are associated on the synaptic vesicle. J. Biol. Chem. 269: 24534-24537.
4. Leimer, U., et al. 1996. Synthesis of the mammalian synaptic vesicle protein synaptophysin in insect cells: a model for vesicle biogenesis. Exp. Cell Res. 224: 88-95.
5. Prekeris, R. and Terrian, D.M. 1997. Brain Myosin V is a synaptic vesicle-associated motor protein: evidence for a Ca^{2+}-dependent interactions with the synaptobrevin-synaptophysin complex. J. Cell Biol. 137: 1589-1601.
6. Becher, A., et al. 1999. The synaptophysin-synaptobrevin complex: a hallmark of synaptic vesicle maturation. J. Neurosci. 19: 1922-1931.

CHROMOSOMAL LOCATION

Genetic locus: Syp (mouse) mapping to X A1.1.

PRODUCT

SYP 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 SYP shRNA Plasmid (m): sc-36596-SH and SYP shRNA (m) Lentiviral Particles: sc-36596-V as alternate gene silencing products.

For independent verification of SYP (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-36596A, sc-36596B and sc-36596C.

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

SYP siRNA (m) is recommended for the inhibition of SYP 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

SYP (D-4): sc-17750 is recommended as a control antibody for monitoring of SYP 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 SYP gene expression knockdown using RT-PCR Primer: SYP (m)-PR: sc-36596-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.