SANTA CRUZ BIOTECHNOLOGY, INC.

VAMP-3 siRNA (m): sc-41339

BACKGROUND

Vesicle-associated membrane proteins, known as VAMPs, also designated synaptobrevins, include VAMP-1, VAMP-2, VAMP-3 (cellubrevin), and synaptotagmin, a protein that may function as an inhibitor of exocytosis. VAMP proteins are vesicular factors that are important components of the machinery controlling docking and/or fusion of secretory vesicles with their target membrane. Synaptosomal-associated proteins, known as SNAPs, including α- and γ-SNAP, are cytoplasmic proteins that bind to a membrane receptor complex composed of VAMP, SNAP 25 and syntaxin. Pancreatic β-cells express VAMP-2 and VAMP-3, and either one or both of these proteins selectively control Ca^{2+}-mediated Insulin secretion. In addition, VAMP-2 and VAMP-3 are expressed on GLUT4-containing vesicle membranes isolated from 3T3-LI adipocytes and are important components of the Insulin-dependent translocation of GLUT4 to the cell surface in adipocytes.

REFERENCES

1. Elferink, L.A., et al. 1993. A role for synaptotagmin (p65) in regulated exocytosis. Cell 72: 153-159.
2. Hayashi, T., et al. 1994. Synaptic vesicle membrane fusion complex: action of clostridial neurotoxins on assembly. EMBO J. 13: 5051-5061.
3. Edelmann, L., et al. 1995. Synaptobrevin binding to synaptophysin: a potential mechanism for controlling the exocytosis fusion machine. EMBO J. 14: 224-231.
4. Regazzi, R., et al. 1995. VAMP-2 and cellubrevin are expressed in pancreatic β-cells and are essential for Ca^{2+}-but not for GTP γ S-induced Insulin secretion. EMBO J. 14: 2723-2730.
5. McMahon, H.T., et al. 1995. Synaptic core complex of synaptobrevin, syntaxin, and SNAP 25 forms high affinity α-SNAP binding site. J. Biol. Chem. 270: 2213-2217.
6. Tamori, Y., et al. 1996. Cleavage of vesicle-associated membrane protein (VAMP)-2 and cellubrevin on GLUT4-containing vesicles inhibits the translocation of GLUT4 in 3T3-L1 adipocytes. Biochem. Biophys. Res. Commun. 220: 740-745.
7. Lin, R.C. and Scheller, R.H. 1997. Structural organization of the synaptic exocytosis core complex. Neuron 19: 1087-1094.

CHROMOSOMAL LOCATION

Genetic locus: Vamp3 (mouse) mapping to 4 E2.

PRODUCT

VAMP-3 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 VAMP-3 shRNA Plasmid (m): sc-41339-SH and VAMP-3 shRNA (m) Lentiviral Particles: sc-41339-V as alternate gene silencing products.

For independent verification of VAMP-3 (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-41339A, sc-41339B and sc-41339C.

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

VAMP-3 siRNA (m) is recommended for the inhibition of VAMP-3 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

VAMP-3 (E-10): sc-514843 is recommended as a control antibody for monitoring of VAMP-3 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 VAMP-3 gene expression knockdown using RT-PCR Primer: VAMP-3 (m)-PR: sc-41339-PR (20 µl, 565 bp). 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.