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SANTA CRUZ BIOTECHNOLOGY, INC.

TI-VAMP siRNA (m): sc-44607

BACKGROUND

Syntaxins were originally thought to be docking proteins, but have more recently been categorized as anchoring proteins that anchor themselves to the cytoplasmic surfaces of cellular membranes. Syntaxins have been shown to bind to various proteins involved in exocytosis, including VAMPs (vesicle-associated membrane proteins, also designated synaptobrevins), NSF (N-ethylmaleimide-sensitive factor), SNAP 25 (synaptosomal-associated protein 25), SNAPs (soluble NSF attachment proteins) and synaptotagmin. Exocytotic vesicles are inserted into the plasma membrane by exocytosis and retrieved by endocytosis. VAMPs are vesicular factors that are important components of the machinery controlling docking and/or fusion of secretory vesicles with their target membrane. Tetanus insensitive VAMP (TI-VAMP) is a type IV membrane protein that is widely expressed. TI-VAMP and cellubrevin form a SNARE complex at the apical plasma membrane. TI-VAMP is insensitive to clostridial neurotoxins.

REFERENCES

1. D'Esposito, M., et al. 1996. A synaptobrevin-like gene in the Xq28 pseudoautosomal region undergoes X inactivation. Nat. Genet. 13: 227-229.
2. Galli, T., et al. 1998. A novel tetanus neurotoxin-insensitive vesicle-associated membrane protein in SNARE complexes of the apical plasma membrane of epithelial cells. Mol. Biol. Cell 9: 1437-1448.
3. Advani, R.J., et al. 1999. VAMP-7 mediates vesicular transport from endosomes to lysosomes. J. Cell Biol. 146: 765-776.
4. Matarazzo, M.R., et al. 1999. Human and mouse SYBL1 gene structure and expression. Gene 240: 233-238.
5. Antonin, W., et al. 2000. A SNARE complex mediating fusion of late endosomes defines conserved properties of SNARE structure and function. EMBO J. 19: 6453-6464.
6. Ward, D.M., et al. 2000. Syntaxin 7 and VAMP-7 are soluble N-ethylmaleimide-sensitive factor attachment protein receptors required for late endosome-lysosome and homotypic lysosome fusion in alveolar macrophages. Mol. Biol. Cell 11: 2327-2333.

CHROMOSOMAL LOCATION

Genetic locus: Vamp7 (mouse) mapping to X.

PRODUCT

TI-VAMP 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 TI-VAMP shRNA Plasmid (m): sc-44607-SH and TI-VAMP shRNA (m) Lentiviral Particles: sc-44607-V as alternate gene silencing products.

For independent verification of TI-VAMP (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-44607A, sc-44607B and sc-44607C.

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

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

TI-VAMP (E-12): sc-166394 is recommended as a control antibody for monitoring of TI-VAMP 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 TI-VAMP gene expression knockdown using RT-PCR Primer: TI-VAMP (m)-PR: sc-44607-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.