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TI-VAMP shRNA (m) Lentiviral Particles: sc-44607-V

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. 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.
4. Matarazzo, M.R., et al. 2002. Allelic inactivation of the pseudoautosomal gene SYBL1 is controlled by epigenetic mechanisms common to the X and Y chromosomes. *Hum. Mol. Genet.* 11: 3191-3198.

CHROMOSOMAL LOCATION

Genetic locus: Vamp7 (mouse) mapping to X.

PRODUCT

TI-VAMP shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 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 TI-VAMP siRNA (m): sc-44607 and TI-VAMP shRNA Plasmid (m): sc-44607-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.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.

APPLICATIONS

TI-VAMP shRNA (m) Lentiviral Particles is recommended for the inhibition of TI-VAMP expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

TI-VAMP (H-55): sc-67060 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.