

## Produktinformation



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# VAMP-2 shRNA (m) Lentiviral Particles: sc-43520-V



The Power to Question

#### **BACKGROUND**

Syntaxins, six of which have been identified, 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), NSF (N-ethylmaleimide-sensitive factor), SNAP 25 (synaptosomal-associated protein of 25 kDa), SNAPs (soluble NSF attachment proteins) and synaptotagmin. VAMPs, also designated synaptobrevins, are vesicular proteins which include VAMP-1, VAMP-2 and synaptotagmin, a protein that may function as an inhibitor of exocytosis. SNAPs, which include  $\alpha$ - and  $\gamma$ -SNAP, are cytoplasmic proteins that bind to a membrane receptor complex composed of VAMP, SNAP 25 and Syntaxin. SNAPs mediate the membrane binding of NSF, which is essential for membrane fusion reactions. An additional protein, synaptophysin, may regulate exocytosis by competing with SNAP 25 and syntaxins for VAMP binding.

#### **REFERENCES**

- Elferink, L.A., et al. 1993. A role for synaptotagmin (p65) in regulated exocytosis. Cell 72: 153-159.
- Bennett, M.K., et al. 1993. The syntaxin family of vesicular transport receptors. Cell 74: 863-873.
- 3. Yamaguchi, K. and Akagawa, K. 1994. Exocytosis relating proteins in the nervous system. Neurosci. Res. 20: 289-292.
- Hayashi, T., et al. 1994. Synaptic vesicle membrane fusion complex: action of clostridial neurotoxins on assembly. EMBO J. 13: 5051-5061.
- Edelmann, L., et al. 1995. Synaptobrevin binding to synaptophysin: a potential mechanism for controlling the exocytosis fusion machine. EMBO J. 14: 224-231.

#### CHROMOSOMAL LOCATION

Genetic locus: Vamp2 (mouse) mapping to 11 B3.

#### **PRODUCT**

VAMP-2 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  $\mu$ l 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 VAMP-2 siRNA (m): sc-43520 and VAMP-2 shRNA Plasmid (m): sc-43520-SH as alternate gene silencing products.

#### **STORAGE**

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

#### **APPLICATIONS**

VAMP-2 shRNA (m) Lentiviral Particles is recommended for the inhibition of VAMP-1 expression in mouse cells.

#### **SUPPORT REAGENTS**

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l 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**

VAMP-2 (3E5): sc-69706 is recommended as a control antibody for monitoring of VAMP-2 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) Immunofluo-rescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

#### **RT-PCR REAGENTS**

Semi-quantitative RT-PCR may be performed to monitor VAMP-2 gene expression knockdown using RT-PCR Primer: VAMP-2 (m)-PR: sc-43520-PR (20  $\mu$ I, 519 bp). 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.

#### **PROTOCOLS**

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