SANTA CRUZ BIOTECHNOLOGY, INC.

VAMP-4 siRNA (h): sc-61766

BACKGROUND

Vesicle-associated membrane protein 4 (VAMP-4) belongs to a subfamily of the large SNARE family. VAMP-4 is distributed mainly in tubular and vesicular membranes of the *trans*-Golgi network, particularly in heart, brain and testis, but is found in almost all tissues. VAMP-4 interacts with small synaptic vesicles and Clathrin-coated vesicles, participating in intracellular trafficking of secreted and membrane-associated proteins. It forms a complex with the TGN-trafficking protein syntaxin 6. VAMP-4 contains a di-leucine motif which binds to the adaptor protein-1 (AP-1) subunit μ-1a. Phosphorylation-dependent binding of the molecule PACS-1 to AP-1 modulates the attachment of AP-1 to VAMP-4. VAMP-4 may contribute to risk for suicide attempt, possibly through alterations in neural conduction.

REFERENCES

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3. Eaton, B.A., et al. 2000. Biogenesis of regulated exocytotic carriers in neuroendocrine cells. J. Neurosci. 20: 7334-7344.
4. Peden, A.A., et al. 2001. The Di-leucine motif of vesicle-associated membrane protein 4 is required for its localization and AP-1 binding. J. Biol. Chem. 276: 49183-491837.
5. Kreykenbohm, V., et al. 2002. The SNAREs vti1a and vti1b have distinct localization and SNARE complex partners. Eur. J. Cell Biol. 81: 273-280.
6. Zeng, Q., et al. 2003. The cytoplasmic domain of VAMP-4 and VAMP-5 is responsible for their correct subcellular targeting: the N-terminal extension of VAMP-4 contains a dominant autonomous targeting signal for the *trans*-Golgi network. J. Biol. Chem. 278: 23046-23054.
7. Hinners, I., et al. 2003. AP-1 recruitment to VAMP-4 is modulated by phosphorylation-dependent binding of PACS-1. EMBO Rep. 4: 1182-1189.
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CHROMOSOMAL LOCATION

Genetic locus: VAMP4 (human) mapping to 1q24.3.

PRODUCT

VAMP-4 siRNA (h) 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-4 shRNA Plasmid (h): sc-61766-SH and VAMP-4 shRNA (h) Lentiviral Particles: sc-61766-V as alternate gene silencing products.

For independent verification of VAMP-4 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-61766A, sc-61766B and sc-61766C.

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-4 siRNA (h) is recommended for the inhibition of VAMP-4 expression in human 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-4 (D-2): sc-365332 is recommended as a control antibody for monitoring of VAMP-4 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-4 gene expression knockdown using RT-PCR Primer: VAMP-4 (h)-PR: sc-61766-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.