SANTA CRUZ BIOTECHNOLOGY, INC.

VAP-A siRNA (m): sc-61769

BACKGROUND

SNAREs are compartmentally specific, integral membrane proteins that are involved in the fusion of membranes and the transport of intracellular proteins. SNAREs are expressed at high levels in all cell types. VAMP-associated protein A (VAP-A) is a SNARE regulator with high levels of expression in the intestine during late embryogenesis and early neonatal development. VAP-A binds to a wide range of SNAREs and fusion-related proteins, including Syntaxin 1A, rBet1, rSec22, αSNAP and NSF. This suggests that VAP-A may play a more general role in SNARE-mediated vesicle traffic between the ER and Golgi in nonpolarized cells. VAP-A also mediates traffic in cell membranes and may play an important role in modulating intestinal smooth muscle cell differentiation. VAP-A and p48 interact to form a stable complex in mammalian cells.

REFERENCES

1. Butler, K.L., et al. 1999. The chest radiograph in critically ill surgical patients is inaccurate in predicting ventilator-associated pneumonia. Am. Surg. 65: 805-809.
2. Nishimura, Y., et al. 1999. Molecular cloning and characterization of mammalian homologues of vesicle-associated membrane protein-associated (VAMP-associated) proteins. Biochem. Biophys. Res. Commun. 254: 21-26.
3. Weir, M.L., et al. 2001. VAP-A binds promiscuously to both v- and tSNAREs. Biochem. Biophys. Res. Commun. 286: 616-621.
4. Wyles, J.P., et al. 2002. Vesicle-associated membrane protein-associated protein-A (VAP-A) interacts with the oxysterol-binding protein to modify export from the endoplasmic reticulum. J. Biol. Chem. 277: 29908-29918.
5. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 605703. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
6. Gabetta, V., et al. 2003. Vesicle-associated protein-A is differentially expressed during intestinal smooth muscle cell differentiation. Dev. Dyn. 228: 11-20.
7. Ettayebi, K. and Hardy, M.E. 2003. Norwalk virus nonstructural protein p48 forms a complex with the SNARE regulator VAP-A and prevents cell surface expression of vesicular stomatitis virus G protein. J. Virol. 77: 11790-11797.

CHROMOSOMAL LOCATION

Genetic locus: Vapa (mouse) mapping to 17 E1.1.

PRODUCT

VAP-A 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 VAP-A shRNA Plasmid (m): sc-61769-SH and VAP-A shRNA (m) Lentiviral Particles: sc-61769-V as alternate gene silencing products.

For independent verification of VAP-A (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-61769A, sc-61769B and sc-61769C.

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

VAP-A siRNA (m) is recommended for the inhibition of VAP-A 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

VAP-A (4C12): sc-293278 is recommended as a control antibody for monitoring of VAP-A 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 VAP-A gene expression knockdown using RT-PCR Primer: VAP-A (m)-PR: sc-61769-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.