

v-SNARE Vti1a siRNA (m): sc-154965

BACKGROUND

Correct vesicular transport is essential to the survival of eukaryotic cells. This process is determined by specific pairing of vesicle-associated SNAREs (v-SNAREs) with those on the target membrane (t-SNAREs). This complex then recruits soluble NSF attachment proteins (SNAPs) and N-ethylmaleimide-sensitive factor (NSF) to form the highly stable SNAP receptor (SNARE) complex. The formation of a SNARE complex pulls the vesicle and target membrane together and may provide the energy to drive fusion of the lipid bilayers. V-SNARE Vti1a (vesicle transport through interaction with t-SNAREs homolog 1A), also known as vesicle transport v-SNARE protein Vti1-like 2, is a 203 amino acid protein that forms a SNARE complex with proteins such as VAMP-3, TI-VAMP, Syntaxin 7, Syntaxin 8 and Syntaxin 10. Levels of v-SNARE Vti1a and Glut4 are decreased with Insulin treatment. Knockdown of v-SNARE Vti1a mRNA inhibits adiponectin secretion and Insulin-stimulated deoxyglucose uptake, suggesting that it may regulate Glut4 and Acpr30 trafficking in adipocytes.

REFERENCES

1. Fischer von Mollard, G. and Stevens, T.H. 1998. A human homolog can functionally replace the yeast vesicle-associated SNARE Vti1p in two vesicle transport pathways. *J. Biol. Chem.* 273: 2624-2630.
2. Bogdanovic, A., et al. 2002. Syntaxin 7, Syntaxin 8, Vti1 and VAMP7 (vesicle-associated membrane protein 7) form an active SNARE complex for early macropinocytic compartment fusion in *Dictyostelium discoideum*. *Biochem. J.* 368: 29-39.
3. Kreykenbohm, V., et al. 2002. The SNAREs Vti1a and Vti1b have distinct localization and SNARE complex partners. *Eur. J. Cell Biol.* 81: 273-280.
4. Bose, A., et al. 2005. The v-SNARE Vti1a regulates Insulin-stimulated glucose transport and Acpr30 secretion in 3T3-L1 adipocytes. *J. Biol. Chem.* 280: 36946-36951.
5. Wang, Y. and Tang, B.L. 2006. SNAREs in neurons—beyond synaptic vesicle exocytosis. *Mol. Membr. Biol.* 23: 377-384.
6. Ganley, I.G., et al. 2008. A syntaxin 10-SNARE complex distinguishes two distinct transport routes from endosomes to the *trans*-Golgi in human cells. *J. Cell Biol.* 180: 159-172.

CHROMOSOMAL LOCATION

Genetic locus: Vti1a (mouse) mapping to 19 D2.

PRODUCT

v-SNARE Vti1a 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see v-SNARE Vti1a shRNA Plasmid (m): sc-154965-SH and v-SNARE Vti1a shRNA (m) Lentiviral Particles: sc-154965-V as alternate gene silencing products.

For independent verification of v-SNARE Vti1a (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-154965A, sc-154965B and sc-154965C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

v-SNARE Vti1a siRNA (m) is recommended for the inhibition of v-SNARE Vti1a 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 μ M in 66 μ l. 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

v-SNARE Vti1a (45): sc-136117 is recommended as a control antibody for monitoring of v-SNARE Vti1a 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 v-SNARE Vti1a gene expression knockdown using RT-PCR Primer: v-SNARE Vti1a (m)-PR: sc-154965-PR (20 μ l). 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.