

USE1 siRNA (m): sc-154939

BACKGROUND

In eukaryotic cells, the Golgi apparatus receives newly synthesized proteins from the endoplasmic reticulum (ER) and, after covalent modification, delivers them to their destination in the cell. For membrane-directed proteins this process is believed to be carried out via vesicular transport. Correct vesicular transport is determined by specific pairing of vesicle-associated SNAREs (v-SNAREs) with those on the target membrane (t-SNAREs). Unconventional SNARE in the ER 1, also known as USE1 or protein p31, is a 259 amino acid t-SNARE that forms a larger complex with ZW10, RINT-1 and Syntaxin 18. Upon Mg^{2+} -AP treatment in the presence of NSF and α -SNAP, ZW10, RINT-1 and USE1 dissociate from Syntaxin 18. USE1 is a single-pass type IV membrane protein that is localized to the endoplasmic reticulum membrane. Three named isoforms exist for USE1 as a result of alternative splicing events.

REFERENCES

- Nichols, B.J. and Pelham, H.R. 1998. SNAREs and membrane fusion in the Golgi apparatus. *Biochim. Biophys. Acta* 1404: 9-31.
- Matsuda, A., Suzuki, Y., Honda, G., Muramatsu, S., Matsuzaki, O., Nagano, Y., Doi, T., Shimotohno, K., Harada, T., Nishida, E., Hayashi, H. and Sugano, S. 2003. Large-scale identification and characterization of human genes that activate NF κ B and MAPK signaling pathways. *Oncogene* 22: 3307-3318.
- Burri, L., Varlamov, O., Doege, C.A., Hofmann, K., Beilharz, T., Rothman, J.E., Söllner, T.H. and Lithgow, T. 2003. A SNARE required for retrograde transport to the endoplasmic reticulum. *Proc. Natl. Acad. Sci. USA* 100: 9873-9877.
- Belgareh-Touzé, N., Corral-Debrinski, M., Launhardt, H., Galan, J.M., Munder, T., Le Panse, S. and Haguenauer-Tsapis, R. 2003. Yeast functional analysis: identification of two essential genes involved in ER to Golgi trafficking. *Traffic* 4: 607-617.
- Hirose, H., Arasaki, K., Dohmae, N., Takio, K., Hatsuzawa, K., Nagahama, M., Tani, K., Yamamoto, A., Tohyama, M. and Tagaya, M. 2004. Implication of ZW10 in membrane trafficking between the endoplasmic reticulum and Golgi. *EMBO J.* 23: 1267-1278.

CHROMOSOMAL LOCATION

Genetic locus: Use1 (mouse) mapping to 8 B3.3.

PRODUCT

USE1 siRNA (m) is a pool of 2 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 USE1 shRNA Plasmid (m): sc-154939-SH and USE1 shRNA (m) Lentiviral Particles: sc-154939-V as alternate gene silencing products.

For independent verification of USE1 (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-154939A and sc-154939B.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

USE1 siRNA (m) is recommended for the inhibition of USE1 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

USE1 (D-1): sc-398316 is recommended as a control antibody for monitoring of USE1 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 USE1 gene expression knockdown using RT-PCR Primer: USE1 (m)-PR: sc-154939-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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