

Exo70 siRNA (h): sc-94143

BACKGROUND

Exocytosis is crucial in membrane trafficking and it mediates hormone and neurotransmitter secretion out of the cell, as well as the incorporation of membrane proteins and lipids to the plasma membrane. It is crucial for cell-cell communication, cell growth and cell polarity. The exocyst complex is a multi-protein complex that consists of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84, and is essential for targeting exocytic vesicles to specific docking sites on the plasma membrane. Exo70, also known as EXOC7 (exocyst complex component 7), EXOC1 or 2-5-3p, is a 735 amino acid peripheral membrane protein that is a component of the exocyst complex. Localized to the cytoplasm and the cell membrane, Exo70 plays an essential role in the docking of exocytic vesicles to target sites on the plasma membrane and, specifically, may be involved in Insulin-induced protein docking within the cell. Four isoforms of Exo70 are expressed due to alternative splicing events.

REFERENCES

1. Kee, Y., et al. 1997. Subunit structure of the mammalian exocyst complex. *Proc. Natl. Acad. Sci. USA* 94: 14438-14443.
2. Kikuno, R., et al. 1999. Prediction of the coding sequences of unidentified human genes. XIV. The complete sequences of 100 new cDNA clones from brain which code for large proteins *in vitro*. *DNA Res.* 6: 197-205.
3. Brymora, A., et al. 2001. The brain exocyst complex interacts with Ral A in a GTP-dependent manner: identification of a novel mammalian Sec3 gene and a second Sec15 gene. *J. Biol. Chem.* 276: 29792-29797.
4. Moskalenko, S., et al. 2003. Ral GTPases regulate exocyst assembly through dual subunit interactions. *J. Biol. Chem.* 278: 51743-51748.
5. Sans, N., et al. 2003. NMDA receptor trafficking through an interaction between PDZ proteins and the exocyst complex. *Nat. Cell Biol.* 5: 520-530.
6. Inoue, M., et al. 2003. The exocyst complex is required for targeting of Glut4 to the plasma membrane by Insulin. *Nature* 422: 629-633.
7. Wang, S., et al. 2004. The mammalian exocyst, a complex required for exocytosis, inhibits tubulin polymerization. *J. Biol. Chem.* 279: 35958-35966.

CHROMOSOMAL LOCATION

Genetic locus: EXOC7 (human) mapping to 17q25.1.

PRODUCT

Exo70 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Exo70 shRNA Plasmid (h): sc-94143-SH and Exo70 shRNA (h) Lentiviral Particles: sc-94143-V as alternate gene silencing products.

For independent verification of Exo70 (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-94143A, sc-94143B and sc-94143C.

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

Exo70 siRNA (h) is recommended for the inhibition of Exo70 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 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

Exo70 (D-6): sc-365825 is recommended as a control antibody for monitoring of Exo70 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 Exo70 gene expression knockdown using RT-PCR Primer: Exo70 (h)-PR: sc-94143-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.