

Sec8 siRNA (m): sc-60086

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. The exocyst complex inhibits tubulin polymerization *in vitro*, suggesting that the exocyst complex is important for modulating the microtubule dynamics that underlie exocytosis. Sec8, also known as EXOC4 (exocyst complex component 4), REC8 or SEC8L1, is one of eight protein subunits composing the mammalian exocyst complex. Human Sec8 maps to chromosome 7q33.

REFERENCES

1. Potenza, M., et al. 1992. Sec6 encodes an 85 kDa soluble protein required for exocytosis in yeast. *Yeast* 8: 549-558.
2. TerBush, D.R., et al. 1995. Sec6, Sec8 and Sec15 are components of a multisubunit complex which localizes to small bud tips in *Saccharomyces cerevisiae*. *J. Cell Biol.* 130: 299-312.
3. Ting, A.E., et al. 1995. rSec6 and rSec8, mammalian homologs of yeast proteins essential for secretion. *Proc. Natl. Acad. Sci. USA* 92: 9613-9617.
4. Friedrich, G.A., et al. 1997. The secretory protein Sec8 is required for paraxial mesoderm formation in the mouse. *Dev. Biol.* 192: 364-374.
5. Grindstaff, K.K., et al. 1998. Sec6/8 complex is recruited to cell-cell contacts and specifies transport vesicle delivery to the basal-lateral membrane in epithelial cells. *Cell* 93: 731-740.
6. Hsu, S.C., et al. 1999. Targeting vesicles to specific sites on the plasma membrane: the role of the Sec6/8 complex. *Trends Cell Biol.* 9: 150-153.
7. Matern, H.T., et al. 2001. The Sec6/8 complex in mammalian cells: characterization of mammalian Sec3, subunit interactions and expression of subunits in polarized cells. *Proc. Natl. Acad. Sci. USA* 98: 9648-9653.
8. Brymora, A., et al. 2001. The brain exocyst complex interacts with RalA in a GTP-dependent manner: identification of a novel mammalian Sec3 gene and a second Sec15 gene. *J. Biol. Chem.* 276: 29792-29797.

CHROMOSOMAL LOCATION

Genetic locus: Exoc4 (mouse) mapping to 6 A3.3.

PRODUCT

Sec8 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 Sec8 shRNA Plasmid (m): sc-60086-SH and Sec8 shRNA (m) Lentiviral Particles: sc-60086-V as alternate gene silencing products.

For independent verification of Sec8 (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-60086A, sc-60086B and sc-60086C.

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

Sec8 siRNA (m) is recommended for the inhibition of Sec8 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

Sec8 (B-11): sc-514215 is recommended as a control antibody for monitoring of Sec8 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 Sec8 gene expression knockdown using RT-PCR Primer: Sec8 (m)-PR: sc-60086-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.