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Sec61 β siRNA (m): sc-155966

BACKGROUND

The Sec61 complex forms the core element of the protein translation complex in the rough endoplasmic reticulum membrane. The complex also associates with two ubiquitous ER membrane proteins: Sec62 (also designated human translocation protein 1 or HTP1) and Sec63. The complex forms a two-way channel that transports proteins both into the ER and back to the cytosol for degradation. Specifically, it appears the β subunit facilitates the escort of proteins back to the cytoplasm for degradation by the proteasome or by other proteolytic systems.

REFERENCES

1. Rapoport, T.A., et al. 1996. Protein transport across the eukaryotic endoplasmic reticulum and bacterial inner membranes. *Annu. Rev. Biochem.* 65: 271-303.
2. Daimon, M., et al. 1997. Identification of a human cDNA homologue to the *Drosophila* translocation protein 1 (Dtrp1). *Biochem. Biophys. Res. Commun.* 230: 100-104.
3. Bebok, Z., et al. 1998. The mechanism underlying cystic fibrosis transmembrane conductance regulator transport from the endoplasmic reticulum to the proteasome includes Sec61 β and a cytosolic, deglycosylated intermediary. *J. Biol. Chem.* 273: 29873-29878.
4. Romisch, K. 1999. Surfing the Sec61 channel: bidirectional protein translocation across the ER membrane. *J. Cell Sci.* 112: 4185-4191.
5. Raden, D., et al. 2000. Role of the cytoplasmic segments of Sec61 α in the ribosome-binding and translocation-promoting activities of the Sec61 complex. *J. Cell Biol.* 150: 53-64.
6. Meyer, H.A., et al. 2000. Mammalian Sec61 is associated with Sec62 and Sec63. *J. Biol. Chem.* 275: 14550-14557.
7. Levy, R., et al. 2001. *In vitro* binding of ribosomes to the β subunit of the Sec61p protein translocation complex. *J. Biol. Chem.* 276: 2340-2346.
8. Alder, N.N., et al. 2005. The molecular mechanisms underlying BiP-mediated gating of the Sec61 translocon of the endoplasmic reticulum. *J. Cell Biol.* 168: 389-399.

CHROMOSOMAL LOCATION

Genetic locus: Sec61b (mouse) mapping to 4 B1.

PRODUCT

Sec61 β 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 Sec61 β shRNA Plasmid (m): sc-155966-SH and Sec61 β shRNA (m) Lentiviral Particles: sc-155966-V as alternate gene silencing products.

For independent verification of Sec61 β (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-155966A and sc-155966B.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20 $^{\circ}$ C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20 $^{\circ}$ 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

Sec61 β siRNA (m) is recommended for the inhibition of Sec61 β 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

Sec61 β (E-6): sc-393633 is recommended as a control antibody for monitoring of Sec61 β 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 MarkerTM 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 Sec61 β gene expression knockdown using RT-PCR Primer: Sec61 β (m)-PR: sc-155966-PR (20 μ l). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ 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.