SANTA CRUZ BIOTECHNOLOGY, INC.

DAD1 siRNA (m): sc-40787

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The Power to Question

BACKGROUND

Membrane proteins of the endoplasmic reticulum (ER) may be localized by mechanisms that involve retention, retrieval, or a combination of both. ER localization information has been found in cytoplasmic, transmembrane or luminal domains. Specific retrieval mechanisms have been identified for luminal ER proteins, which contain a KDEL domain, and for type I transmembrane proteins carrying a dilysine motif. The mammalian oligosaccharyltransferase (OST) is a protein complex that is composed of four rough ER-specific, type I transmembrane proteins: ribophorins I and II (RI and RII), OST48, and DAD1 (also designated defender against apoptotic death). The ribophorins are integral membrane glycoproteins that localize exclusively to the rough endoplasmic reticulum. There is affinity between the cytoplasmically located N-terminal region of DAD1 and the short cytoplasmic tail of OST48 to place DAD1 firmly into the OST complex. The OST affects the cotranslational N-glycosylation of newly synthesized polypeptides.

REFERENCES

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2. Fu, J., Ren, M. and Kreibich, G. 1997. Interactions among subunits of the oligosaccharyltransferase complex. J. Biol. Chem. 272: 29687-29692.
3. Kelleher, D.J. and Gilmore, R. 1997. DAD1, the defender against apoptotic cell death, is a subunit of the mammalian oligosaccharyltransferase. Proc. Natl. Acad. Sci. USA 94: 4994-4999.
4. Sanjay, A., Fu, J. and Kreibich, G. 1998. DAD1 is required for the function and the structural integrity of the oligosaccharyltransferase complex. J. Biol. Chem. 273: 26094-26099.
5. Fu, J., Pirozzi, G., Sanjay, A., Levy, R., Chen, Y., De Lemos-Chiarandini, C., Sabatini, D. and Kreibich, G. 2000. Localization of Ribophorin II to the endoplasmic reticulum involves both its transmembrane and cytoplasmic domains. Eur. J. Cell Biol. 79: 219-228.
6. Fu, J. and Kreibich, G. 2000. Retention of subunits of the oligosaccharyltransferase complex in the endoplasmic reticulum. J. Biol. Chem. 275: 3984-3990.

CHROMOSOMAL LOCATION

Genetic locus: Dad1 (mouse) mapping to 14 C2.

PRODUCT

DAD1 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see DAD1 shRNA Plasmid (m): sc-40787-SH and DAD1 shRNA (m) Lentiviral Particles: sc-40787-V as alternate gene silencing products.

For independent verification of DAD1 (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-40787A, sc-40787B and sc-40787C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

DAD1 siRNA (m) is recommended for the inhibition of DAD1 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 µM in 66 µl. 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor DAD1 gene expression knockdown using RT-PCR Primer: DAD1 (m)-PR: sc-40787-PR (20 µl, 347 bp). 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.