SANTA CRUZ BIOTECHNOLOGY, INC.

ALG9 siRNA (m): sc-141017

BACKGROUND

Glycosylation of asparagine residues is an essential protein modification reaction that occurs upon most proteins that enter the secretory pathway in eukaryotic cells. Asparagine-linked oligosaccharides are transferred onto polypeptides in the lumen of the rough endoplasmic reticulum. ALG9 (asparagine-linked glycosylation 9, α-1,2-mannosyltransferase homolog), also known as DIBD1, is a 611 amino acid multi-pass membrane protein that localizes to the endoplasmic reticulum. Ubiquitously expressed, with highest levels in heart, liver and pancreas, ALG9 catalyzes the transfer of mannose from Dol-P-Man to lipid-linked oligosaccharides. Defects in the gene encoding ALG9 may be caused by congenital disorder of glycosylation type 1L (CDG1L). CDGs are a family of severe inherited diseases, including sychomotor retardation, dysmorphic features, hypotonia, coagulation disorders and immunodeficiency, caused by a defect in protein N-glycosylation and are characterized by under-glycosylated serum proteins. Four isoforms of ALG9 exist due to alternative splicing events.

REFERENCES

1. de L Ufret, M., et al. 2000. Probing the extended binding determinants of oligosaccharyl transferase with synthetic inhibitors of asparagine-linked glycosylation. Bioorg. Med. Chem. Lett. 10: 281-284.
2. Nagayama, Y., et al. 2000. Identification of the sites of asparagine-linked glycosylation on the human thyrotropin receptor and studies on their role in receptor function and expression. J. Pharmacol. Exp. Ther. 295: 404-409.
3. Uchimura, S., et al. 2005. Effects of N-glycosylation and inositol on the ER stress response in yeast *Saccharomyces cerevisiae.* Biosci. Biotechnol. Biochem. 69: 1274-1280.
4. Bickel, T., et al. 2005. Biosynthesis of lipid-linked oligosaccharides in *Saccharomyces cerevisiae:* Alg13p and Alg14p form a complex required for the formation of GlcNAc(2)-PP-dolichol. J. Biol. Chem. 280: 34500-34506.
5. Gao, X.D., et al. 2005. ALG14 recruits ALG13 to the cytoplasmic face of the endoplasmic reticulum to form a novel bipartite UDP-N-acetylglucosamine transferase required for the second step of N-linked glycosylation. J. Biol. Chem. 280: 36254-36262.
6. Averbeck, N., et al. 2007. Membrane topology of the ALG14 endoplasmic reticulum UDP-GlcNAc transferase subunit. J. Biol. Chem. 282: 29081-29088.

CHROMOSOMAL LOCATION

Genetic locus: Alg9 (mouse) mapping to 9 A5.3.

PRODUCT

ALG9 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 ALG9 shRNA Plasmid (m): sc-141017-SH and ALG9 shRNA (m) Lentiviral Particles: sc-141017-V as alternate gene silencing products.

For independent verification of ALG9 (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-141017A, sc-141017B and sc-141017C.

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

ALG9 siRNA (m) is recommended for the inhibition of ALG9 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 ALG9 gene expression knockdown using RT-PCR Primer: ALG9 (m)-PR: sc-141017-PR (20 µl). 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.