SANTA CRUZ BIOTECHNOLOGY, INC.

ALG6 siRNA (h): sc-88385

BACKGROUND

ALG6 (asparagine-linked glycosylation 6 homolog), is a 507 amino acid member of the ALG6/ALG8 glucosyltransferase family that functions as an α1,3-glucosyltransferase required for proper asparagine-linked glycosylation of proteins. ALG6 is a multi-pass membrane protein that localizes to the endoplasmic reticulum (ER). Specifically, ALG6 adds the first of three glucose residues added to dolichylpyrophosphate-linked oligosaccharide, a precursor for N-linked glycosylation. Mutations in the gene encoding ALG6 disrupt protein glycosylation and result in congenital disorder of glycosylation type 1C (CDG1C). CDG1C is a multisystem disease characterized by underglycosylated serum proteins. Patients with CDG1C exhibit delayed statomotor development, are mentally retarded and have muscular hypotonia.

REFERENCES

1. Imbach, T., et al. 1999. A mutation in the human ortholog of the *Saccharomyces cerevisiae* ALG6 gene causes carbohydrate-deficient glycoprotein syndrome type-1C. Proc. Natl. Acad. Sci. USA 96: 6982-6987.
2. Westphal, V., et al. 2000. Reduced heparan sulfate accumulation in enterocytes contributes to protein-losing enteropathy in a congenital disorder of glycosylation. Am. J. Pathol. 157: 1917-1925.
3. Westphal, V., et al. 2000. Analysis of multiple mutations in the hALG6 gene in a patient with congenital disorder of glycosylation 1C. Mol. Genet. Metab. 70: 219-223.
4. Freeze, H.H. and Westphal, V. 2001. Balancing N-linked glycosylation to avoid disease. Biochimie 83: 791-799.
5. Oriol, R., et al. 2002. Common origin and evolution of glycosyltransferases using Dol-P-monosaccharides as donor substrate. Mol. Biol. Evol. 19: 1451-1463.
6. Westphal, V., et al. 2003. Identification of a frequent variant in ALG6, the cause of congenital disorder of glycosylation-Ic. Hum. Mutat. 22: 420-421.
7. Newell, J.W., et al. 2003. Congenital disorder of glycosylation Ic in patients of Indian origin. Mol. Genet. Metab. 79: 221-228.

CHROMOSOMAL LOCATION

Genetic locus: ALG6 (human) mapping to 1p31.3.

PRODUCT

ALG6 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ALG6 shRNA Plasmid (h): sc-88385-SH and ALG6 shRNA (h) Lentiviral Particles: sc-88385-V as alternate gene silencing products.

For independent verification of ALG6 (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-88385A, sc-88385B and sc-88385C.

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

ALG6 siRNA (h) is recommended for the inhibition of ALG6 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 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.

GENE EXPRESSION MONITORING

ALG6 (Z164): sc-100506 is recommended as a control antibody for monitoring of ALG6 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 ALG6 gene expression knockdown using RT-PCR Primer: ALG6 (h)-PR: sc-88385-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.