SANTA CRUZ BIOTECHNOLOGY, INC.

ALG12 siRNA (m): sc-141012

BACKGROUND

ALG12 (asparagine-linked glycosylation 12 homolog), also known as ECM39 or membrane protein SB87, is a 488 amino acid member of the glycosyltransferase 22 family that functions as a mannosyltransferase required for proper protein glycosylation. ALG12 is a multi-pass membrane protein that is expressed in fibroblasts and localizes to the endoplasmic reticulum (ER). Specifically, ALG12 catalyzes the addition of α1,6 mannose to dolichol-linked Man7GlcNAc2. Defects in ALG12 disrupt protein N-glycosylation and result in congenital disorder of glycosylation type 1G (CDG1G). CDG1G is a multi-system disease characterized by under-glycosylated serum proteins. N-glycoproteins play important roles in cell maintenance, embryonic development and differentiation. A disease affecting the proper function of these proteins can lead to coagulation disorders, psychomotor retardation, hypotonia, immunodeficiency and dysmorphic features.

REFERENCES

1. Burda, P., et al. 1999. Ordered assembly of the asymmetrically branched lipid-linked oligosaccharide in the endoplasmic reticulum is ensured by the substrate specificity of the individual glycosyltransferases. Glycobiology 9: 617-625.
2. Grimme, S.J., et al. 2001. The essential SMP3 protein is required for addition of the side-branching fourth mannose during assembly of yeast glycosylphosphatidylinositols. J. Biol. Chem. 276: 27731-27739.
3. Grubenmann, C.E., et al. 2002. ALG12 mannosyltransferase defect in congenital disorder of glycosylation type Ig. Hum. Mol. Genet. 11: 2331-2339.
4. Chantret, I., et al. 2002. Congenital disorders of glycosylation type Ig is defined by a deficiency in dolichyl-P-mannose:Man7GlcNAc2-PP-dolichyl mannosyltransferase. J. Biol. Chem. 277: 25815-25822.
5. Zdebska, E., et al. 2003. Abnormal glycosylation of red cell membrane band 3 in the congenital disorder of glycosylation Ig. Pediatr. Res. 54: 224-229.

CHROMOSOMAL LOCATION

Genetic locus: Alg12 (mouse) mapping to 15 E3.

PRODUCT

ALG12 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 ALG12 shRNA Plasmid (m): sc-141012-SH and ALG12 shRNA (m) Lentiviral Particles: sc-141012-V as alternate gene silencing products.

For independent verification of ALG12 (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-141012A, sc-141012B and sc-141012C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

ALG12 siRNA (m) is recommended for the inhibition of ALG12 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.

GENE EXPRESSION MONITORING

ALG12 (Q16): sc-100507 is recommended as a control antibody for monitoring of ALG12 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 ALG12 gene expression knockdown using RT-PCR Primer: ALG12 (m)-PR: sc-141012-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.