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Glucosidase I siRNA (m): sc-145445

BACKGROUND

Glycosylation of asparagine residues in Asn-X-Ser/Thr motifs in proteins commonly occur in the lumen of the endoplasmic reticulum (ER). Glucosidase I catalyzes the first step in the N-linked oligosaccharide processing pathway. It specifically removes the distal α 1,2-linked glucose residue from the Glc3-Man9-GlcNAc2 oligosaccharide precursor. Glucosidase I contains a short cytosolic tail, a single pass transmembrane domain and a large C-terminal catalytic domain located on the luminal side of the ER. Mutations in the gene encoding Glucosidase I result in the congenital disorder glycosylation (CDG-IIb), which is characterized by generalized hypotonia, dysmorphic features, hepatomegaly, hypoventilation, feeding problems, seizures and death. Two point mutations in the Glucosidase I gene have been identified and result in amino acid substitutions, namely Arg486Thr and Phe652Leu, that affect polypeptide folding and active site formation.

REFERENCES

1. Kalz-Füller, B., Bieberich, E. and Bause, E. 1995. Cloning and expression of glucosidase I from human hippocampus. *Eur. J. Biochem.* 231: 344-351.
2. Khan, F.A., et al. 1999. Genomic organization and promoter activity of glucosidase I gene. *Glycobiology* 9: 797-806.
3. De Praeter CM, G.J., et al. 2000. A novel disorder caused by defective biosynthesis of N-linked oligosaccharides due to glucosidase I deficiency. *Am. J. Hum. Genet.* 66: 1744-1756.
4. Völker, C., et al. 2002. Processing of N-linked carbohydrate chains in a patient with glucosidase I deficiency (CDG type IIb). *Glycobiology* 12: 473-483.
5. Hardt, B., et al. 2003. (Arg)3 within the N-terminal domain of glucosidase I contains ER targeting information but is not required absolutely for ER localization. *Glycobiology* 13: 159-168.
6. Hong, Y., et al. 2004. The Lec23 Chinese hamster ovary mutant is a sensitive host for detecting mutations in α -glucosidase I that give rise to congenital disorder of glycosylation IIb (CDG IIb). *J. Biol. Chem.* 279: 49894-49901.
7. Ruddock, L.W., et al. 2006. N-glycan processing in ER quality control. *J. Cell Sci.* 119: 4373-4380.

CHROMOSOMAL LOCATION

Genetic locus: Mogs (mouse) mapping to 6 C3.

PRODUCT

Glucosidase I 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 Glucosidase I shRNA Plasmid (m): sc-145445-SH and Glucosidase I shRNA (m) Lentiviral Particles: sc-145445-V as alternate gene silencing products.

For independent verification of Glucosidase I (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-145445A and sc-145445B.

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 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

Glucosidase I siRNA (m) is recommended for the inhibition of Glucosidase I 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

Glucosidase I (C-11): sc-374006 is recommended as a control antibody for monitoring of Glucosidase I 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 Marker[™] 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 Glucosidase I gene expression knockdown using RT-PCR Primer: Glucosidase I (m)-PR: sc-145445-PR (20 μ l). 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.