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SANTA CRUZ BIOTECHNOLOGY, INC.

ERGIC-53 siRNA (m): sc-45247

BACKGROUND

Lectin mannose-binding 1, also designated vesicular integral-membrane protein (VIP36) and lectin mannose-binding 2, also designated ER-Golgi intermediate compartment (ERGIC-53) comprise a family of membrane bound, ubiquitous proteins involved in the selective transport of newly synthesized glycoproteins from the endoplasmic reticulum (ER) to the ER-Golgi intermediate compartment (ERGIC). VIP36 acts as an intracellular lectin in the early secretory pathway. It is involved in the sorting and transport of glycoproteins carrying high mannose-type glycans. ERGIC-53, a mannose-specific lectin, recognizes sugar residues of glycoproteins and glycolipids. It mediates the sorting and recycling of proteins and/or lipids. Null expression of ERGIC-53, also designated LMAN1, results in a rare autosomal recessive bleeding disorder that causes combined deficiency of both coagulation factors V and VIII.

REFERENCES

1. Schindler, R., et al. 1993. ERGIC-53, a membrane protein of the ER-Golgi intermediate compartment, carries an ER retention motif. Eur. J. Cell Biol. 61: 1-9.
2. Kappeler, F., et al. 1994. A dual role for COOH-terminal lysine residues in pre-Golgi retention and endocytosis of ERGIC-53. J. Biol. Chem. 269: 6279-6281.
3. Hauri, H.P., et al. 2002. Lectins and protein traffic early in the secretory pathway. Biochem. Soc. Symp. 69: 73-82.
4. Cunningham, M.A., et al. 2003. LMAN1 is a molecular chaperone for the secretion of coagulation factor VIII. J. Thromb. Haemost. 1: 2360-2367.
5. Hara-Kuge, S., et al. 2004. The binding of VIP36 and α-Amylase in the secretory vesicles via high mannose-type glycans. Glycobiology 14: 739-744.
6. Kamiya, Y., et al. 2005. Sugar-binding properties of VIP36, an intracellular animal lectin operating as a cargo receptor. J. Biol. Chem. 280: 37178-37182.
7. Neve, E.P., et al. 2005. Oligomerization and interacellular localization of the glycoprotein receptor ERGIC-53 is independent of disulfide bonds. J. Mol. Biol. 354: 556-568.

CHROMOSOMAL LOCATION

Genetic locus: Lman1 (mouse) mapping to 18 E1.

PRODUCT

ERGIC-53 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 ERGIC-53 shRNA Plasmid (m): sc-45247-SH and ERGIC-53 shRNA (m) Lentiviral Particles: sc-45247-V as alternate gene silencing products.

For independent verification of ERGIC-53 (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-45247A, sc-45247B and sc-45247C.

STORAGE AND RESUSPENSION

Store lyophilized shRNA plasmid DNA at 4° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at 4° C for short term storage or -80° C for long term storage. Avoid repeated freeze thaw cycles.

Resuspend lyophilized shRNA plasmid DNA in 200 µl of the deionized water provided. Resuspension of the shRNA plasmid DNA in 200 µl of deionized water makes a 0.1 µg/µl solution in a 10 mM Tris, 1 mM EDTA buffered solution.

APPLICATIONS

ERGIC-53 siRNA (m) is recommended for the inhibition of ERGIC-53 expression in mouse cells.

SUPPORT REAGENTS

For optimal shRNA Plasmid transfection efficiency, Santa Cruz Biotechnology's shRNA Plasmid Transfection Reagent: sc-108061 (0.2 ml) and shRNA Plasmid Transfection Medium: sc-108062 (20 ml) are recommended. Control shRNAs are available as 20 µg lyophilized plasmid DNA. Each encodes a scrambled shRNA sequence that will not lead to the specific degradation of any known cellular mRNA. Control shRNA Plasmids include: sc-108060, sc-108065 and sc-108066.

GENE EXPRESSION MONITORING

ERGIC-53 (F-3): sc-398777 is recommended as a control antibody for monitoring of ERGIC-53 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 ERGIC-53 gene expression knockdown using RT-PCR Primer: ERGIC-53 (m)-PR: sc-45247-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.