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ERGIC-53 siRNA (h): sc-45246

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

- 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.
- 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.
- Hauri, H.P., et al. 2002. Lectins and protein traffic early in the secretory pathway. *Biochem. Soc. Symp.* 69: 73-82.
- Cunningham, M.A., et al. 2003. LMAN1 is a molecular chaperone for the secretion of coagulation factor VIII. *J. Thromb. Haemost.* 1: 2360-2367.
- 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.

CHROMOSOMAL LOCATION

Genetic locus: LMAN1 (human) mapping to 18q21.32.

PRODUCT

ERGIC-53 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ERGIC-53 shRNA Plasmid (h): sc-45246-SH and ERGIC-53 shRNA (h) Lentiviral Particles: sc-45246-V as alternate gene silencing products.

For independent verification of ERGIC-53 (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-45246A, sc-45246B and sc-45246C.

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.

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

ERGIC-53 siRNA (h) is recommended for the inhibition of ERGIC-53 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 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

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-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 ERGIC-53 gene expression knockdown using RT-PCR Primer: ERGIC-53 (h)-PR: sc-45246-PR (20 μ l, 513 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.