

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

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- 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.
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- 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 μ M 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 μ l of the deionized water provided. Resuspension of the shRNA plasmid DNA in 200 μ l of deionized water makes a 0.1 μ g/ μ l 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 μ g 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-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 (m)-PR: sc-45247-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.