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ERGIC-53 shRNA (m) Lentiviral Particles: sc-45247-V

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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- 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.
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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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CHROMOSOMAL LOCATION

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

PRODUCT

ERGIC-53 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see ERGIC-53 siRNA (m): sc-45247 and ERGIC-53 shRNA Plasmid (m): sc-45247-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80°C . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

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

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

ERGIC-53 (A-18): sc-32442 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

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