

MCFD2 siRNA (m): sc-44446

BACKGROUND

Multiple coagulation factor deficiency protein 2 (MCFD2) is localized in the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) through a direct, calcium-dependent interaction with LMAN1. The MCFD2-LMAN1 complex forms a specific cargo receptor for the transport of selected proteins from the endoplasmic reticulum to the Golgi apparatus. Mutations in the MCFD2 gene may cause of Factor V and Factor VIII combined deficiency (F5F8D). F5F8D is an autosomal recessive human bleeding disorder characterized by the reduction of both clotting proteins.

REFERENCES

1. Zhang, B., et al. 2003. Bleeding due to disruption of a cargo-specific ER-to-Golgi transport complex. *Nat. Genet.* 34: 220-225.
2. Spatuzza, C., et al. 2004. Heat shock induces preferential translation of ERGIC-53 and affects its recycling pathway. *J. Biol. Chem.* 279: 42535-42544.
3. Zhang, B., et al. 2004. Familial multiple coagulation factor deficiencies: new biologic insight from rare genetic bleeding disorders. *J. Thromb. Haemost.* 2: 1564-1572.
4. Zhang, B., et al. 2005. LMAN1 and MCFD2 form a cargo receptor complex and interact with coagulation Factor VIII in the early secretory pathway. *J. Biol. Chem.* 280: 25881-25886.
5. Mohanty, D., et al. 2005. Mutations in the MCFD2 gene and a novel mutation in the LMAN1 gene in Indian families with combined deficiency of Factor V and VIII. *Am. J. Hematol.* 79: 262-266.
6. Zhang, B., et al. 2006. Combined deficiency of factor V and factor VIII is due to mutations in either LMAN1 or MCFD2. *Blood* 107: 1903-1907.

CHROMOSOMAL LOCATION

Genetic locus: *Mcf2* (mouse) mapping to 17 E4.

PRODUCT

MCFD2 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 MCFD2 shRNA Plasmid (m): sc-44446-SH and MCFD2 shRNA (m) Lentiviral Particles: sc-44446-V as alternate gene silencing products.

For independent verification of MCFD2 (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-44446A, sc-44446B and sc-44446C.

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

MCFD2 siRNA (m) is recommended for the inhibition of MCFD2 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

MCFD2 (F-3): sc-390463 is recommended as a control antibody for monitoring of MCFD2 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 MCFD2 gene expression knockdown using RT-PCR Primer: MCFD2 (m)-PR: sc-44446-PR (20 μ l, 575 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.