SANTA CRUZ BIOTECHNOLOGY, INC.

GM130 siRNA (m): sc-41225

BACKGROUND

The docking of transport vesicles to their target membrane is mediated by p115. GM130, a *cis*-Golgi matrix protein, interacts specifically with p115 and provides a membrane docking site. Both GM130 and p115 are involved in vesicle tethering to Golgi membranes. The amino-terminus of GM130 binds to p115, whereas the carboxy-terminus binds to Golgi membranes. Both Giantin and GM130 compete for binding to p115. Thus, p115-Giantin and p115-GM130 interactions might mediate independent membrane tethering events. Transport from the ER to the *cis*/medial Golgi compartments requires the action of p115, GM130 and Giantin via a sequential rather than a coordinate mechanism. Mitotic phosphorylation of GM130 at Serine 25 is mediated by Cdc2, prevents binding to p115 and is directly involved in mitotic Golgi fragmentation. GM130 is phosphorylated in prophase as the Golgi complex starts to break down, and remains phosphorylated in metaphase and anaphase. In telophase, GM130 is dephosphorylated by PP2A as the Golgi fragments start to reassemble.

REFERENCES

1. Nakamura, N., et al. 1997. The vesicle docking protein p115 binds GM130, a *cis*-Golgi matrix protein, in a mitotically regulated manner. Cell 89: 445-455.
2. Lowe, M., et al. 1998. Cdc2 kinase directly phosphorylation the *cis*-Golgi matrix protein GM130 and is required for Golgi fragmentation in mitosis. Cell 94: 783-793.
3. Linstedt, A.D., et al. 2000. Binding relationships of membrane tethering components. The Giantin N-terminus and the GM130 N-terminus compete for binding to the p115 C-terminus. J. Biol. Chem. 275: 10196-10201.
4. Alvarez, C.I., et al. 2000. The p115-interactive proteins, GM130 and Giantin participate in ER-Golgi traffic. J. Biol. Chem. 276: 2693-2700.

CHROMOSOMAL LOCATION

Genetic locus: Golga2 (mouse) mapping to 2 B.

PRODUCT

GM130 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 GM130 shRNA Plasmid (m): sc-41225-SH and GM130 shRNA (m) Lentiviral Particles: sc-41225-V as alternate gene silencing products.

For independent verification of GM130 (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-41225A, sc-41225B and sc-41225C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

GM130 siRNA (m) is recommended for the inhibition of GM130 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 µM in 66 µl. 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

GM130 (H-7): sc-55590 is recommended as a control antibody for monitoring of GM130 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 GM130 gene expression knockdown using RT-PCR Primer: GM130 (m)-PR: sc-41225-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Casey, C.A., et al. 2016. Study of ethanol-induced Golgi disorganization reveals the potential mechanism of alcohol-impaired N-glycosylation. Alcohol. Clin. Exp. Res. 40: 2573-2590.

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.