SANTA CRUZ BIOTECHNOLOGY, INC.

GBF1 siRNA (m): sc-145349

BACKGROUND

Protein trafficking to the membrane requires formation of coated carrier vesicles, such as COPI-coated vesicles from the *cis*-Golgi, a process triggered by membrane binding of the GTP-bound form of ADP-ribosylation factors. Normally, brefeldin A (BFA) blocks this action by inhibiting guanine nucleotide exchange factors (GEFs) for ADP-ribosylation factor. However, GBF1, a member of the Sec7-domain family of GEFs, allows cells to maintain normal golgi morphology and grow in the presence of BFA. The gene encoding the human GBF1 protein maps to chromosome 10q24, with the sec7-domain centrally positioned, and a proline-rich C-terminus. Based on mutatagenesis analysis, this proline rich region appears to interact with p115 in a functionally significant manner.

REFERENCES

1. Mansour, S.J., et al. 1998. Human GBF1 is a ubiquitously expressed gene of the Sec7 domain family mapping to 10q24. Genomics 54: 323-327.
2. Claude, A., et al. 1999. GBF1: a novel Golgi-associated BFA-resistant guanine nucleotide exchange factor that displays specificity for ADP-ribosylation factor 5. J. Cell Biol. 146: 71-84.
3. Kawamoto, K., et al. 2002. GBF1, a guanine nucleotide exchange factor for ADP-ribosylation factors, is localized to the *cis*-Golgi and involved in membrane association of the COPI coat. Traffic 3: 483-495.
4. Garcia-Mata, R., et al 2003. The membrane-tethering protein p115 interacts with GBF1, an ARF guanine-nucleotide-exchange factor. EMBO Rep. 4: 320-325.
5. Garcia-Mata, R., et al. 2003. ADP-ribosylation factor/COPI-dependent events at the endoplasmic reticulum-Golgi interface are regulated by the guanine nucleotide exchange factor GBF1. Mol. Biol. Cell 14: 2250-2261.
6. Niu, T.K., et al. 2005. Dynamics of GBF1, a brefeldin A-sensitive ARF1 exchange factor at the Golgi. Mol. Biol. Cell 16:1213-1222.

CHROMOSOMAL LOCATION

Genetic locus: Gbf1 (mouse) mapping to 19 C3.

PRODUCT

GBF1 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 GBF1 shRNA Plasmid (m): sc-145349-SH and GBF1 shRNA (m) Lentiviral Particles: sc-145349-V as alternate gene silencing products.

For independent verification of GBF1 (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-145349A, sc-145349B and sc-145349C.

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 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

GBF1 siRNA (m) is recommended for the inhibition of GBF1 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

GBF1 (25): sc-136240 is recommended as a control antibody for monitoring of GBF1 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 GBF1 gene expression knockdown using RT-PCR Primer: GBF1 (m)-PR: sc-145349-PR (20 µl). 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.