

GGA3 siRNA (h): sc-41171

BACKGROUND

The GGA family of proteins (Golgi-localized, γ -Adaptin ear-containing, ARF-binding proteins) are ubiquitous coat proteins that facilitate the trafficking of soluble proteins from the *trans*-Golgi network (TGN) to endosomes/lysosomes by means of interactions with TGN-sorting receptors, ARF (ADP-ribosylation factor), and clathrin. Members of the GGA family, GGA1, GGA2 (also known as VEAR) and GGA3, are multi-domain proteins that bind mannose 6-phosphate receptors (MPRs). GGAs have modular structures with an N-terminal VHS (VPS27, Hrs and STAM) domain followed by a GAT (GGA and Tom1) domain, a connecting hinge segment and a C-terminal GAE (γ -Adaptin ear) domain. The amino-terminal VHS domains of GGAs form complexes with the cytoplasmic domains of sorting receptors by recognizing acidic-cluster dileucine (ACLL) sequences. The human GGA3 gene maps to chromosome 17 and encodes a 723 amino acid protein that shares 46% sequence identity with GGA1 and 38% with GGA2.

REFERENCES

- Hirst, J., Lui, W.W., Bright, N.A., Totty, N., Seaman, M.N. and Robinson, M.S. 2000. A family of proteins with γ -Adaptin and VHS domains that facilitate trafficking between the *trans*-Golgi network and the vacuole/lysosome. *J. Cell Biol.* 149: 67-80.
- Dell'Angelica, E.C., Puertollano, R., Mullins, C., Aguilar, R.C., Vargas, J.D., Hartnell, L.M. and Bonifacio, J.S. 2000. GGAs: a family of ADP ribosylation factor-binding proteins related to adaptors and associated with the Golgi complex. *J. Cell Biol.* 149: 81-94.
- Shiba, T., Takatsu, H., Nogi, T., Matsugaki, N., Kawasaki, M., Igarashi, N., Suzuki, M., Kato, R., Earnest, T., Nakayama, K. and Wakatsuki, S. 2002. Structural basis for recognition of acidic-cluster dileucine sequence by GGA1. *Nature* 415: 937-941.
- Doray, B., Ghosh, P., Griffith, J., Geuze, H.J. and Kornfeld, S. 2002. Cooperation of GGAs and AP-1 in packaging MPRs at the *trans*-Golgi network. *Science* 297: 1700-1703.
- Doray, B., Bruns, K., Ghosh, P. and Kornfeld, S.A. 2002. Autoinhibition of the ligand-binding site of GGA1/3 VHS domains by an internal acidic cluster-dileucine motif. *Proc. Natl. Acad. Sci. USA* 99: 8072-8077.
- Takatsu, H., Yoshino, K., Toda, K. and Nakayama, K. 2002. GGA proteins associate with Golgi membranes through interaction between their GGAH domains and ADP-ribosylation factors. *Biochem. J.* 365: 369-378.

CHROMOSOMAL LOCATION

Genetic locus: GGA3 (human) mapping to 17q25.1.

PRODUCT

GGA3 siRNA (h) is a target-specific 19-25 nt siRNA 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 GGA3 shRNA Plasmid (h): sc-41171-SH and GGA3 shRNA (h) Lentiviral Particles: sc-41171-V as alternate gene silencing 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

GGA3 siRNA (h) is recommended for the inhibition of GGA3 expression in human 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

GGA3 (8): sc-135923 is recommended as a control antibody for monitoring of GGA3 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 MarkerTM 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 GGA3 gene expression knockdown using RT-PCR Primer: GGA3 (h)-PR: sc-41171-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.