SANTA CRUZ BIOTECHNOLOGY, INC.

GGA1 siRNA (h): sc-41167

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The Power to Question

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 multidomain proteins that bind mannose 6-phosphate receptors (MPRs). GGAs have modular structures with an N-terminal VHS (VPS-27, 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. GGA1 and GGA2 do not associate with each other, but they do colocalize on perinuclear membranes. The cytosolic domain of memapsin 2, but not that of memapsin 1, binds the VHS domains of GGA1 and GGA2. The human GGA1 gene maps to chromosome 22q13.1 and encodes a protein that shares 45% sequence identity with GGA2 and GGA3.

REFERENCES

1. Hirst, J., et al. 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.
2. Shiba, T., et al. 2002. Structural basis for recognition of acidic-cluster dileucine sequence by GGA1. Nature 415: 937-941.
3. Doray, B., et al. 2002. Cooperation of GGAs and AP-1 in packaging MPRs at the *trans*-Golgi network. Science 297: 1700-1703.
4. Doray, B., et al. 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.
5. He, X., et al. 2002. Memapsin 2 (β-secretase) cytosolic domain binds to the VHS domains of GGA1 and GGA2: implications on the endocytosis mechanism of memapsin 2. FEBS Lett. 524: 183-187.
6. LocusLink Report (LocusID: 606004). http://www.ncbi.nlm.nih.gov/LocusLink/

CHROMOSOMAL LOCATION

Genetic locus: GGA1 (human) mapping to 22q13.1.

PRODUCT

GGA1 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GGA1 shRNA Plasmid (h): sc-41167-SH and GGA1 shRNA (h) Lentiviral Particles: sc-41167-V as alternate gene silencing products.

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

GGA1 siRNA (h) is recommended for the inhibition of GGA1 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 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

GGA1 (D-6): sc-271927 is recommended as a control antibody for monitoring of GGA1 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 GGA1 gene expression knockdown using RT-PCR Primer: GGA1 (h)-PR: sc-41167-PR (20 µl, 599 bp). 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.