SANTA CRUZ BIOTECHNOLOGY, INC.

ARFGAP1 siRNA (h): sc-72529

BACKGROUND

ARFGAP1 (ADP-ribosylation factor GTPase activating protein 1), also known as ARF1 GAP, is a 406 amino acid protein that contains one ARF-GAP domain and localizes to the cytoplasm and the Golgi apparatus. Functioning as a GTPase-activating protein (GAP), ARFGAP1 interacts with ARF1 and promotes hydrolysis of ARF1-bound GTP, an event that is required for both the dissociation of coat proteins from Golgi structures and for the subsequent fusion of Golgi vesicles with target compartments. When overexpressed, ARFGAP1, whose activity is inhibited by phosphatidylcholine and stimulated by phosphoinosides, can induce the redistribution of the entire Golgi apparatus to the endoplasmic reticulum. Multiple isoforms of ARFGAP1 exist due to alternative splicing events.

REFERENCES

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2. Zhang, C., et al. 2000. Characterization, chromosomal assignment, and tissue expression of a novel human gene belonging to the ARF GAP family. Genomics 63: 400-408.
3. Majoul, I., et al. 2001. KDEL-cargo regulates interactions between proteins involved in COPI vesicle traffic: measurements in living cells using FRET. Dev. Cell 1: 139-153.
4. Yang, J.S., et al. 2002. ARFGAP1 promotes the formation of COPI vesicles, suggesting function as a component of the coat. J. Cell Biol. 159: 69-78.
5. Bernards, A., et al. 2004. GAP control: regulating the regulators of small GTPases. Trends Cell Biol. 14: 377-385.
6. Parnis, A., et al. 2006. Golgi localization determinants in ArfGAP1 and in new tissue-specific ArfGAP1 isoforms. J. Biol. Chem. 281: 3785-3792.
7. Natsume, W., et al. 2006. SMAP2, a novel ARF GTPase-activating protein, interacts with clathrin and clathrin assembly protein and functions on the AP-1-positive early endosome/*trans*-Golgi network. Mol. Biol. Cell 17: 2592-2603.

CHROMOSOMAL LOCATION

Genetic locus: ARFGAP1 (human) mapping to 20q13.33.

PRODUCT

ARFGAP1 siRNA (h) 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 ARFGAP1 shRNA Plasmid (h): sc-72529-SH and ARFGAP1 shRNA (h) Lentiviral Particles: sc-72529-V as alternate gene silencing products.

For independent verification of ARFGAP1 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-72529A, sc-72529B and sc-72529C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

ARFGAP1 siRNA (h) is recommended for the inhibition of ARFGAP1 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

ARFGAP1 (C-4): sc-271303 is recommended as a control antibody for monitoring of ARFGAP1 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 ARFGAP1 gene expression knockdown using RT-PCR Primer: ARFGAP1 (h)-PR: sc-72529-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.