

ARFGAP1/3 siRNA (h): sc-60200

BACKGROUND

G protein-coupled receptor kinases (GRKs) are activated by activated G protein-coupled receptors, and they function to phosphorylate and inactivate cell surface receptors in the heterotrimeric G protein signaling cascade. GIT1 (for GRK-interactor 1) and GIT2 are GTPase-activating proteins (GAPs) for members of the ADP ribosylation factor (ARF) family of small GTP-binding proteins, which are involved in vesicular trafficking. Another member of the ARF family, the cytoplasmic ARFGAP (ADP-ribosylation factor GTPase-activating protein) 1/3 protein, is involved in the dissociation of coat proteins from Golgi-derived membranes and vesicles. ARFGAP1/3, a cytoplasmic protein localizing to the perinuclear region, plays a role in protein secretion and vesicle transport and promotes hydrolysis of GTP bound to ARF-1. The activity of the ARFGAP1/3 protein is phospholipid sensitive. It is primarily expressed in endocrine glands and testis, but is also highly expressed in adult brain, thymus and lung.

REFERENCES

1. 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.
2. Liu, X., et al. 2001. Functional characterization of novel human ARFGAP3. *FEBS Lett.* 490: 79-83.
3. Turner, C.E., et al. 2001. Paxillin-ARF GAP signaling and the cytoskeleton. *Curr. Opin. Cell Biol.* 13: 593-539.
4. Collins, J.E., et al. 2004. A genome annotation-driven approach to cloning the human ORFeome. *Genome Biol.* 5: R84.
5. Yoon, H.Y., et al. 2004. Differences between AGAP1, ASAP1 and Arf GAP1 in substrate recognition: interaction with the N-terminus of Arf1. *Cell. Signal.* 16: 1033-1044.

CHROMOSOMAL LOCATION

Genetic locus: ARFGAP1 (human) mapping to 20q13.33, ARFGAP3 (human) mapping to 22q13.2.

PRODUCT

ARFGAP1/3 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ARFGAP1/3 shRNA Plasmid (h): sc-60200-SH and ARFGAP1/3 shRNA (h) Lentiviral Particles: sc-60200-V as alternate gene silencing products.

For independent verification of ARFGAP1/3 (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-60200A, sc-60200B and sc-60200C.

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 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

ARFGAP1/3 siRNA (h) is recommended for the inhibition of ARFGAP1/3 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

ARFGAP1/3 (D-8): sc-365418 is recommended as a control antibody for monitoring of ARFGAP1/3 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 Marker™ 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 ARFGAP1/3 gene expression knockdown using RT-PCR Primer: ARFGAP1/3 (h)-PR: sc-60200-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Castoldi, F., et al. 2020. Autophagy-mediated metabolic effects of aspirin. *Cell Death Discov.* 6: 129.

RESEARCH USE

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