

# ARFGAP1/3 siRNA (m): sc-60201

## 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.
6. SWISS-PROT/TrEMBL (Q9NP61). World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>

## CHROMOSOMAL LOCATION

Genetic locus: Arfgap1 (mouse) mapping to 2 H4, Arfgap3 (mouse) mapping to 15 E1.

## PRODUCT

ARFGAP1/3 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ARFGAP1/3 shRNA Plasmid (m): sc-60201-SH and ARFGAP1/3 shRNA (m) Lentiviral Particles: sc-60201-V as alternate gene silencing products.

For independent verification of ARFGAP1/3 (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-60201A, sc-60201B and sc-60201C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

ARFGAP1/3 siRNA (m) is recommended for the inhibition of ARFGAP1/3 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  $\mu$ M in 66  $\mu$ 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 (m)-PR: sc-60201-PR (20  $\mu$ l). 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](http://www.scbt.com) for detailed protocols and support products.