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ARF6 siRNA (m): sc-43620

BACKGROUND

The ADP-ribosylation factor (ARF) protein family are structurally and functionally conserved members of the Ras superfamily of regulatory GTP-binding proteins. ARFs influence vesicle trafficking and signal transduction in eukaryotic cells. ARF-dependent regulatory mechanisms include the coordination of spectrin interactions with Golgi membranes and the association of Actin to the Golgi via Rho family-dependent G protein localization (Rac, Cdc42) and WASP/Arp2/3 complexes. Additionally, ARFs play a central role in maintenance of organelle integrity, assembly of coat proteins and activation of phospholipase D. The ARF proteins are categorized as class I (ARF1, ARF2 and ARF3), class II (ARF4 and ARF5) and class III (ARF6); members of each class share a common gene organization. The human ARF6 gene contains five exons and four introns, and encodes a 175 amino acid protein.

REFERENCES

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2. Amor, J.C., Harrison, D.H., Kahn, R.A. and Ringe, D. 1994. Structure of the human ADP-ribosylation factor 1 complexed with GDP. *Nature* 372: 704-708.
3. Erickson, J.W., Zhang, C., Khan, R.A., Evans, T. and Cerione, R.A. 1996. Mammalian Cdc42 is a brefeldin A-sensitive component of the Golgi apparatus. *J. Biol. Chem.* 271: 26850-26854.
4. Godi, A., Santone, I., Pertile, P., Devarajan, P., Stabach, P.R., Morrow, J.S., Di Tullio, G., Polishchuk, R., Petrucci, T.C., Luini, A. and De Matteis, M.A. 1998. ADP ribosylation factor regulates spectrin binding to the Golgi complex. *Proc. Natl. Acad. Sci. USA* 95: 8607-8612.

CHROMOSOMAL LOCATION

Genetic locus: Arf6 (mouse) mapping to 12 C2.

PRODUCT

ARF6 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ARF6 shRNA Plasmid (m): sc-43620-SH and ARF6 shRNA (m) Lentiviral Particles: sc-43620-V as alternate gene silencing products.

For independent verification of ARF6 (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-43620A, sc-43620B and sc-43620C.

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

ARF6 siRNA (m) is recommended for the inhibition of ARF6 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 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

ARF6 (3A-1): sc-7971 is recommended as a control antibody for monitoring of ARF6 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 ARF6 gene expression knockdown using RT-PCR Primer: ARF6 (m)-PR: sc-43620-PR (20 μ l, 595 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.

PROTOCOLS

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