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ARF6 shRNA (h) Lentiviral Particles: sc-43619-V

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

1. Randazzo, P.A., et al. 1994. The amino-terminus of ADP-ribosylation factor (ARF) 1 is essential for interaction with G_s and ARF GTPase-activating protein. *J. Biol. Chem.* 269: 29490-29494.
2. Amor, J.C., et al. 1994. Structure of the human ADP-ribosylation factor 1 complexed with GDP. *Nature* 372: 704-708.
3. Erickson, J.W., et al. 1996. Mammalian Cdc42 is a brefeldin A-sensitive component of the Golgi apparatus. *J. Biol. Chem.* 271: 26850-26854.
4. Godi, A., et al. 1998. ADP ribosylation factor regulates spectrin binding to the Golgi complex. *Proc. Natl. Acad. Sci. USA* 95: 8607-8612.

CHROMOSOMAL LOCATION

Genetic locus: ARF6 (human) mapping to 14q21.3.

PRODUCT

ARF6 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 2 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see ARF6 siRNA (h): sc-43619 and ARF6 shRNA Plasmid (h): sc-43619-SH as alternate gene silencing products.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

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

APPLICATIONS

ARF6 siRNA (h) is recommended for the inhibition of ARF6 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ARF6 gene expression knockdown using RT-PCR Primer: ARF6 (h)-PR: sc-43619-PR (20 μ l, 521 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.