

ARF siRNA (h): sc-29725

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.

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.
5. Fucini, R.V., et al. 2000. Activated ADP-ribosylation factor assembles distinct pools of actin on Golgi membranes. *J. Biol. Chem.* 275: 18824-18829.
6. Fucini, R.V., et al. 2002. Golgi vesicle proteins are linked to the assembly of an actin complex defined by mAbp1. *Mol. Biol. Cell* 13: 621-631.

PRODUCT

ARF siRNA (h) is a pool of 5 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 ARF shRNA Plasmid (h): sc-29725-SH and ARF shRNA (h) Lentiviral Particles: sc-29725-V as alternate gene silencing products.

For independent verification of ARF (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-29725A, sc-29725B and sc-29725C, sc-29725D and sc-29725E.

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

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

ARF (ARF3 3F1): sc-53167 is recommended as a control antibody for monitoring of ARF 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.

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.