SANTA CRUZ BIOTECHNOLOGY, INC.

ACAP2 siRNA (m): sc-60122

BACKGROUND

The ADP-ribosylation factor (ARF) family of small GTP-binding proteins are involved in vesicular transport regulation and in controlling cytoskeletal organization and cell adhesion. These proteins mainly regulate membrane traffic. ACAP2 is a member of the centaurin GTPase-activating protein (GAP) family, which comprises a subset of ARF regulatory molecules that transduce PI 3-kinase activation into coordinated control of ARF-dependent pathways. ACAP1 and ACAP2 are both widely expressed in peripheral, tubular membranes and usually interact with each other in various tissues. GAP activity of both ACAP1 and ACAP2 is dependent upon phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P2]. ACAP2 associates with ARF1 and ARF6. Overexpression of ACAP2 blocks the formation of ARF6-dependent protrusions. K1L is a protein required for growth of the Vaccinia Virus that interacts with the ankyrin repeats of ACAP2.

REFERENCES

1. Jackson, T.R., et al. 2000. ACAPs are ARF6 GTPase-activating proteins that function in the cell periphery. J. Cell Biol. 151: 627-638.
2. Furman, C., et al. 2002. DEF-1/ASAP1 is a GTPase-activating protein (GAP) for ARF1 that enhances cell motility through a GAP-dependent mechanism. J. Biol. Chem. 277: 7962-7969.
3. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 607766. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
4. Jovanovic, O.A., et al. 2005. An effector domain mutant of ARF6 implicates phospholipase D in endosomal membrane recycling. Mol. Biol. Cell 17: 327-335.
5. Thacker, E., et al. 2005. The ARF6 GAP Centaurin α1 is a neuronal Actin-binding protein which also functions via GAP-independent activity to regulate the Actin cytoskeleton. Eur. J. Cell Biol. 83: 541-554.
6. Bradley, R.R. and Terajima, M. 2005. Vaccinia Virus K1L protein mediates host-range function in RK-13 cells via ankyrin repeat and may interact with a cellular GTPase-activating protein. Virus Res. 114: 104-112.
7. Gizachew, D., et al. 2006. NMR structural studies of the myristoylated N-terminus of ADP ribosylation factor 6 (ARF6). FEBS Lett. 580: 4296-4301.

CHROMOSOMAL LOCATION

Genetic locus: Acap2 (mouse) mapping to 16 B2.

PRODUCT

ACAP2 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ACAP2 shRNA Plasmid (m): sc-60122-SH and ACAP2 shRNA (m) Lentiviral Particles: sc-60122-V as alternate gene silencing products.

For independent verification of ACAP2 (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-60122A, sc-60122B and sc-60122C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

ACAP2 siRNA (m) is recommended for the inhibition of ACAP2 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 µM in 66 µl. 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

ACAP2 (F-8): sc-376150 is recommended as a control antibody for monitoring of ACAP2 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 ACAP2 gene expression knockdown using RT-PCR Primer: ACAP2 (m)-PR: sc-60122-PR (20 µl). 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.