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SANTA CRUZ BIOTECHNOLOGY, INC.

ACAP1 siRNA (m): sc-45741

BACKGROUND

ACAP1, also designated Centaurin-β 1 (CENTB1 or Cnt-b1), is a member of the ADP ribosylation factor family of Arf6 GTPase-activating proteins (GAP). GAPs are important regulators of Arf function by controlling the return of Arf to its inactive state. ACAP1 is related to AGAP1 and ASAP1, and all three proteins are similarly expressed in fibroblast cells such as NIH/3T3. Internalization and recycling of integrin receptors is important in cell adhesion and migration modulation, and once inside a cell, proteins and membranes are transported to the endosome where they are sorted for recycling or degradation. ACAP1 promotes cargo sorting by associating directly to recycling cargo proteins. Preventing this interaction inhibits protein recycling. ACAP1 binds transferrin receptors, promoting their transport to the plasma membrane from the endosome. Akt induced phosphorylation of ACAP1 at Ser 554 regulates ACAP1 interaction to integrin in endosomes, and downregulation of Akt or ACAP1 may inhibit cell migration on Fibronectin.

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. Nie, Z., et al. 2003. Specific regulation of the adaptor protein complex AP-3 by the Arf GAP AGAP1. Dev. Cell 5: 513-521.
4. Dai, J., et al. 2004. ACAP1 promotes endocytic recycling by recognizing recycling sorting signals. Dev. Cell 7: 771-776.
5. Ivaska, J., et al. 2005. PKCε-mediated phosphorylation of Vimentin controls integrin recycling and motility. EMBO J. 24: 3834-3845.
6. Li, J., et al. 2005. Phosphorylation of ACAP1 by Akt regulates the stimulation-dependent recycling of Integrin β1 to control cell migration. Dev. Cell 9: 663-673.

CHROMOSOMAL LOCATION

Genetic locus: Centb1 (mouse) mapping to 11 B3.

PRODUCT

ACAP1 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 ACAP1 shRNA Plasmid (m): sc-45741-SH and ACAP1 shRNA (m) Lentiviral Particles: sc-45741-V as alternate gene silencing products.

For independent verification of ACAP1 (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-45741A, sc-45741B and sc-45741C.

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 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

ACAP1 siRNA (m) is recommended for the inhibition of ACAP1 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

ACAP1 (G-4): sc-137172 is recommended as a control antibody for monitoring of ACAP1 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 ACAP1 gene expression knockdown using RT-PCR Primer: ACAP1 (m)-PR: sc-45741-PR (20 µl). 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 for detailed protocols and support products.