SANTA CRUZ BIOTECHNOLOGY, INC.

CdGAP siRNA (m): sc-142222

BACKGROUND

The superfamily of GTP-binding proteins, for which the Ras proteins are prototypes, has been implicated in regulation of diverse biological activities involving various aspects of cell growth and division. Cdc42 mediates many signaling pathways that lead to transcriptional activation, cell cycle control and Actin reorganization. CdGAP (Cdc42 GTPase-activating protein) is a 1,444 amino acid protein that serves as a GAP (GTP-activating protein) for the Rho GTPases Cdc42 and RAC1, but not RhoA. Overexpression of CdGAP in Cos-7 cells results in membrane blebbing, suggesting that CdGAP may play a role in apoptosis. Via binding to GSK-3α and GSK-3β, human CdGAP is phosphorylated on theronine 776. CdGAP is ubiquitously expressed in all tissues with highest levels in muscle and heart.

REFERENCES

1. Lamarche-Vane, N., et al. 1998. CdGAP, a novel proline-rich GTPase-activating protein for Cdc42 and Rac. J. Biol. Chem. 273: 29172-29177.
2. Jenna, S., et al. 2002. The activity of the GTPase-activating protein CdGAP is regulated by the endocytic protein intersectin. J. Biol. Chem. 277: 6366-6373.
3. Itoh, R.E., et al. 2002. Activation of rac and Cdc42 video imaged by fluorescent resonance energy transfer-based single-molecule probes in the membrane of living cells. Mol. Cell. Biol. 22: 6582-6591.
4. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 610911. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. Tcherkezian, J., et al. 2005. Extracellular signal-regulated kinase 1 interacts with and phosphorylates CdGAP at an important regulatory site. Mol. Cell. Biol. 25: 6314-6329.
6. Tcherkezian, J., et al. 2006. The human orthologue of CdGAP is a phosphoprotein and a GTPase-activating protein for Cdc42 and Rac1 but not RhoA. Biol. Cell 98: 445-456.
7. LaLonde, D.P., et al. 2006. CdGAP associates with actopaxin to regulate integrin-dependent changes in cell morphology and motility. Curr. Biol. 16: 1375-1385.

CHROMOSOMAL LOCATION

Genetic locus: Arhgap31 (mouse) mapping to 16 B4.

PRODUCT

CdGAP 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 CdGAP shRNA Plasmid (m): sc-142222-SH and CdGAP shRNA (m) Lentiviral Particles: sc-142222-V as alternate gene silencing products.

For independent verification of CdGAP (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-142222A, sc-142222B and sc-142222C.

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

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

CdGAP (G-8): sc-393839 is recommended as a control antibody for monitoring of CdGAP 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 CdGAP gene expression knockdown using RT-PCR Primer: CdGAP (m)-PR: sc-142222-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.