SANTA CRUZ BIOTECHNOLOGY, INC.

Rac GAP1 siRNA (m): sc-76336

BACKGROUND

A large number of low molecular weight, GTP binding proteins of the Ras superfamily have been identified. These proteins regulate many fundamental processes in all eukaryotic cells such as growth, vesicle traffic and cytoskeletal organization. GTPase-activating proteins (GAPs) accelerate the intrinsic rate of GTP hydrolysis of Ras-related proteins, resulting in downregulation of their active form. Through this function, GAPs negatively regulate Ras-mediated signaling. Rac GAP1 (Rac GTPase activating protein 1), also known as MgcRacGAP (male germ cell Rac GTPase activating protein), ID-GAP or HsCYK-4, functions as a GAP and exhibits strong activity towards Rac 1 and Cdc42. Highly expressed in thymus, placenta and testis with lower levels in spleen and peripheral blood lymphocytes, Rac GAP1 contains one Rho-GAP domain and one phorbol-ester/DAG-type zinc finger. Rac GAP1 plays an essential role in cytokinesis, functioning as a scaffold protein as well as a GTPase regulator. During cytokinesis, Rac GAP1 is phosphorylated at multiple sites.

REFERENCES

1. Toure, A., et al. 1998. MgcRacGAP, a new human GTPase-activating protein for Rac and Cdc42 similar to *Drosophila* RotundRacGAP gene product, is expressed in male germ cells. J. Biol. Chem. 273: 6019-6023.
2. Toure, A., et al. 2001. TAT1, a novel sulfate transporter specifically expressed in human male germ cells and potentially linked to Rho GTPase signaling. J. Biol. Chem. 276: 20309-20315.
3. Hu, Y., et al. 2002. Molecular characterization of a metastatic neuroendocrine cell cancer arising in the prostates of transgenic mice. J. Biol. Chem. 277: 44462-44474.
4. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 604980. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. Lu, K.H., et al. 2004. Selection of potential markers for epithelial ovarian cancer with gene expression arrays and recursive descent partition analysis. Clin. Cancer Res. 10: 3291-3300.
6. Katoh, Y. and Katoh, M. 2004. Identification and characterization of ARHGAP27 gene in silico. Int. J. Mol. Med. 14: 943-947.

CHROMOSOMAL LOCATION

Genetic locus: Racgap1 (mouse) mapping to 15 F1.

PRODUCT

Rac GAP1 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 Rac GAP1 shRNA Plasmid (m): sc-76336-SH and Rac GAP1 shRNA (m) Lentiviral Particles: sc-76336-V as alternate gene silencing products.

For independent verification of Rac GAP1 (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-76336A, sc-76336B and sc-76336C.

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

Rac GAP1 siRNA (m) is recommended for the inhibition of Rac GAP1 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

Rac GAP1 (A-6): sc-271110 is recommended as a control antibody for monitoring of Rac GAP1 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 Rac GAP1 gene expression knockdown using RT-PCR Primer: Rac GAP1 (m)-PR: sc-76336-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.