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SANTA CRUZ BIOTECHNOLOGY, INC.

Rap1GAP siRNA (r): sc-270196

BACKGROUND

Rap1 GTPase activating protein (Rap1GAP) specifically stimulates GTP hydrolytic activity of the monomeric G protein Rap1. Physical interaction between $G_{\alpha z}$, a member of the G_i family of trimeric G proteins, and Rap1GAP blocks the ability of regulators of G protein signaling to stimulate GTP hydrolysis of the α subunit, and also attenuates the ability of activated $G_{\alpha z}$ to inhibit adenylyl cyclase. Rap1GAP is expressed in the brain, kidney and pancreas and may act as a signal integrator to coordinate and/or integrate G_z signaling and Rap1 signaling in cells. A novel isoform of Rapl GTPase-activating protein, designated Rap1GAPII, binds specifically to $G_{\alpha z}$. Stimulation of the G_i-coupled M2 muscarinic receptor translocates Rap1GAPII from the cytosol to the membrane and decreases the amount of GTP-bound Rap1, resulting in the activation of ERK/MAPK.

REFERENCES

1. Janoueix-Lerosey, I., et al. 1994. Phosphorylation of Rap1GAP during the cell cycle. Biochem. Biophys. Res. Commun. 202: 967-975.
2. Wada, Y., et al. 1997. Mitogen-inducible SIPA1 is mapped to the conserved syntenic groups of chromosome 19 in mouse and chromosome 11q13.3 centromeric to BCL1 in human. Genomics 39: 66-73.
3. Kurachi, H., et al. 1997. Human SPA-1 gene product selectively expressed in lymphoid tissues is a specific GTPase-activating protein for Rap1 and Rap2. Segregate expression profiles from a Rap1GAP gene product. J. Biol. Chem. 272: 28081-28088.
4. Jordan, J.D., et al. 1999. Modulation of Rap activity by direct interaction of $G_{\alpha o}$ with Rap1 GTPase-activating protein. J. Biol. Chem. 274: 21507-21510.
5. Meng, J., et al. 1999. Functional interaction between $G_{\alpha z}$ and Rap1GAP suggests a novel form of cellular cross-talk. J. Biol. Chem. 274: 36663-36669.
6. Mochizuki, N., et al. 1999. Activation of the ERK/MAPK pathway by an isoform of Rap1GAP associated with G_{α}. Nature 400: 891-894.

CHROMOSOMAL LOCATION

Genetic locus: Rap1gap (rat) mapping to 5q36.

PRODUCT

Rap1GAP siRNA (r) 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 Rap1GAP shRNA Plasmid (r): sc-270196-SH and Rap1GAP shRNA (r) Lentiviral Particles: sc-270196-V as alternate gene silencing products.

For independent verification of Rap1GAP (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270196A, sc-270196B and sc-270196C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

Rap1GAP siRNA (r) is recommended for the inhibition of Rap1GAP expression in rat 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

Rap1GAP (D-9): sc-166586 is recommended as a control antibody for monitoring of Rap1GAP 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 Rap1GAP gene expression knockdown using RT-PCR Primer: Rap1GAP (r)-PR: sc-270196-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.