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SANTA CRUZ BIOTECHNOLOGY, INC.

GAP1^{m} siRNA (h): sc-41704

BACKGROUND

Ras p21 can exist in either a physiologically quiescent GDP-binding state or a GTP-binding signal-emitting state. Interaction of Ras p21 with GTPase activating protein (GAP) can increase the rate of hydrolysis of Ras p21-bound GTP by as much as 1,000-fold. In mitogenically activated and tyrosine kinase-transformed cells, Ras GAP forms a complex with a protein designated p190. At its amino terminus, p190 contains sequence motifs characteristic of all known GTPases, whereas the carboxy terminus contains sequences similar to those found in the Bcr gene product, n-chimerin and Rho GAP, all of which exhibit intrinsic GAP activity. GAP1^{m} is an additional protein with GTPase activating activity. GAP1^{m} contains a GAP catalytic domain, a phospholipid-binding region and a domain that shares homology with a unique domain of Btk. GAP1^{m} is most highly expressed in brain, placenta and kidney.

REFERENCES

1. Barbacid, M. 1987. Ras genes. Annu. Rev. Biochem. 56: 779-827.
2. Trahey, M. and McCormick, F. 1987. A cytoplasmic protein stimulates normal N-Ras p21 GTPase, but does not affect oncogenic mutants. Science 242: 1697-1700.
3. Sanders, D.A. 1990. A guide to the low molecular weight GTPases. Cell Growth Differ. 1: 251-258.
4. Bourne, H.R., Sanders, D.A. and McCormick, F. 1990. The GTPase superfamily: a conserved switch for diverse cell functions. Nature 348: 125-132.
5. Settleman, J., Narasimhan, V., Foster, L.C. and Weinberg, R.A. 1992. Molecular cloning of cDNAs encoding the GAP-associated protein p190: implications for a signaling pathway from Ras to the nucleus. Cell 69: 539-549.
6. Maekawa, M., Li, S., Iwamatsu, A., Morishita, T., Yokota, K., Imai, Y., Kohsaka, S., Nakamura, S. and Hattori, S. 1994. A novel mammalian Ras GTPase-activating protein which has phospholipid-binding and Btk homology regions. Mol. Cell. Biol. 14: 6879-6885.

CHROMOSOMAL LOCATION

Genetic locus: RASA2 (human) mapping to 3q23.

PRODUCT

GAP1^{m} siRNA (h) 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 GAP1^{m} shRNA Plasmid (h): sc-41704-SH and GAP1^{m} shRNA (h) Lentiviral Particles: sc-41704-V as alternate gene silencing products.

For independent verification of GAP1^{m} (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-41704A, sc-41704B and sc-41704C.

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

GAP1^{m} siRNA (h) is recommended for the inhibition of GAP1^{m} expression in human 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

GAP1^{m} (15): sc-135916 is recommended as a control antibody for monitoring of GAP1^{m} 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 GAP1^{m} gene expression knockdown using RT-PCR Primer: GAP1^{m} (h)-PR: sc-41704-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.