

C3G siRNA (m): sc-29864

BACKGROUND

Ras p21 is the prototype of a superfamily of GTPases that is involved in the regulation of a wide variety of cellular processes. Ras signals in its GTP-bound form but is "turned off" when bound to GDP. When unregulated or constitutively turned on by mutations, Ras signaling contributes to malignant transformation. The switch between active and inactive Ras is controlled by GTPase-activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs). C3G was isolated in a screen for proteins that could bind the SH3 domain of the Crk proto-oncogene product. The carboxy-terminus of the C3G protein displays significant sequence similarity to Ras-GRF/Cdc25Mm and mSos and can substitute for Cdc25 function in *S. cerevisiae*. These observations strongly suggest that C3G is a GEF for Ras and is involved in the regulation of Ras signaling through Crk. The C3G gene maps to human chromosome 9q34.13 in proximity to the gene that encodes c-Abl, a proto-oncogene that regulates Crk.

REFERENCES

1. Barbacid, M. 1987. Ras genes. *Annu. Rev. Biochem.* 56: 779-827.
2. Boguski, M.S., et al. 1993. Proteins regulating Ras and its relatives. *Nature* 366: 643-654.
3. Tanaka, S., et al. 1994. C3G, a guanine nucleotide-releasing protein expressed ubiquitously, binds to the Src homology 3 domains of CRK and GRB2/ASH proteins. *Proc. Natl. Acad. Sci. USA* 91: 3443-3447.
4. Matsuda, M., et al. 1994. CRK protein binds to two guanine nucleotide-releasing proteins for the Ras family and modulates nerve growth factor-induced activation of Ras in PC12 cells. *Mol. Cell. Biol.* 14: 5495-5500.
5. Takai, S., et al. 1994. Mapping of the human C3G gene coding a guanine nucleotide releasing protein for Ras family to 9q34.3 by fluorescence *in situ* hybridization. *Hum. Genet.* 94: 549-550.

CHROMOSOMAL LOCATION

Genetic locus: Rapgef1 (mouse) mapping to 2 B.

PRODUCT

C3G 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see C3G shRNA Plasmid (m): sc-29864-SH and C3G shRNA (m) Lentiviral Particles: sc-29864-V as alternate gene silencing products.

For independent verification of C3G (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-29864A, sc-29864B and sc-29864C.

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.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

C3G siRNA (m) is recommended for the inhibition of C3G 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 μ M in 66 μ l. 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

C3G (G-4): sc-17840 is recommended as a control antibody for monitoring of C3G 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 C3G gene expression knockdown using RT-PCR Primer: C3G (m)-PR: sc-29864-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.