

Ran GAP1 siRNA (h): sc-37159

BACKGROUND

The small Ras related protein Ran, also called TC4, is a nuclear localized GTPase implicated in a diverse array of cellular processes including DNA replication, entry into and exit from mitosis and the transport of RNA and proteins through the nuclear pore complex. Like Ras, active Ran GTP and inactive Ran GDP levels are tightly regulated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). The abundant GEF RCC1 (regulator of chromosome condensation 1) increases the rate at which Ran exchanges GDP for GTP. Ran GAP1 opposes the effects of RCC1 by increasing the rate at which Ran hydrolyzes GTP to GDP. A protein designated Ran BP1 has no intrinsic GAP activity and functions as a GEF inhibitor deactivating RCC1 and thereby indirectly increasing the ratio of Ran GDP to Ran GTP. Ran BP2 has been proposed as the Ran GTP docking site at the periphery of the nuclear pore complex.

REFERENCES

1. Scheffzek, K., et al. 1995. Crystal structure of the nuclear Ras-related protein Ran in its GDP-bound form. *Nature* 374: 378-381.
2. Beddow, A.L., et al. 1995. The Ran/TC4 GTPase-binding domain: identification by expression cloning and characterization of a conserved sequence motif. *Proc. Natl. Acad. Sci. USA* 92: 3328-3332.
3. Ren, M., et al. 1995. Separate domains of the Ran GTPase interact with different factors to regulate nuclear protein import and RNA processing. *Mol. Cell. Biol.* 15: 2117-2124.
4. Bischoff, F.R., et al. 1995. Co-activation of Ran GTPase and inhibition of GTP dissociation by Ran-GTP binding protein Ran BP1. *EMBO J.* 14: 705-715.
5. Klebe, C., et al. 1995. Interaction of the nuclear GTP-binding protein Ran with its regulatory proteins RCC1 and Ran GAP1. *Biochemistry* 34: 639-647.
6. Moroianu, J., et al. 1995. Protein export from the nucleus requires the GTPase Ran and GTP hydrolysis. *Proc. Natl. Acad. Sci. USA* 92: 4318-4322.
7. Melchior, F., et al. 1995. GTP hydrolysis by Ran occurs at the nuclear pore complex in an early step of protein import. *J. Cell Biol.* 131: 571-581.

CHROMOSOMAL LOCATION

Genetic locus: RANGAP1 (human) mapping to 22q13.2.

PRODUCT

Ran GAP1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Ran GAP1 shRNA Plasmid (h): sc-37159-SH and Ran GAP1 shRNA (h) Lentiviral Particles: sc-37159-V as alternate gene silencing products.

For independent verification of Ran GAP1 (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-37159A, sc-37159B and sc-37159C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

Ran GAP1 siRNA (h) is recommended for the inhibition of Ran GAP1 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 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

Ran GAP1 (C-5): sc-28322 is recommended as a control antibody for monitoring of Ran 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-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 Ran GAP1 gene expression knockdown using RT-PCR Primer: Ran GAP1 (h)-PR: sc-37159-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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