

Rag B siRNA (m): sc-152684

BACKGROUND

The Ras-related superfamily of guanine nucleotide binding proteins includes the R-Ras, Rap, Ral/Rec and Rho/Rab subfamilies, all of which are thought to play an important role in either endocytosis or in biosynthetic protein transport. The process of transporting newly synthesized proteins from the endoplasmic reticulum (ER) to various stacks of the Golgi complex and to secretory vesicles involves the movement of carrier vesicles and requires Rab protein function. Rag B (Ras-related GTP-binding protein B), also known as RRAGB, is a 374 amino acid cytoplasmic protein that belongs to the GTR/RAG GTP-binding protein family. Existing as a short and long form due to alternative splicing events, Rag B's short isoform binds GTP. Rag B has guanine nucleotide-binding activity and is required for amino acid-induced mTORC1 relocalization. Rag B is also involved in the activation of RHEB, a GTPase, and the subsequent activation of the TOR signaling cascade.

REFERENCES

- Huber, L.A., et al. 1994. Mapping of Ras-related GTP-binding proteins by GTP overlay following two-dimensional gel electrophoresis. *Proc. Natl. Acad. Sci. USA* 91: 7874-7878.
- Schürmann, A., et al. 1995. Cloning of a novel family of mammalian GTP-binding proteins (RagA, RagBs, RagB1) with remote similarity to the Ras-related GTPases. *J. Biol. Chem.* 270: 28982-28988.
- Hirose, E., et al. 1998. RagA is a functional homologue of *S. cerevisiae* Gtr1p involved in the Ran/Gsp1-GTPase pathway. *J. Cell Sci.* 111: 11-21.
- Segikuchi, T., et al. 2001. Novel G proteins, Rag C and Rag D, interact with GTP-binding proteins, Rag A and Rag B. *J. Biol. Chem.* 276: 7246-7257.
- Sancak, Y., et al. 2008. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science* 320: 1496-1501.
- Sancak, Y., et al. 2010. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell* 141: 290-303.

CHROMOSOMAL LOCATION

Genetic locus: *Ragb* (mouse) mapping to X F3.

PRODUCT

Rag B 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 Rag B shRNA Plasmid (m): sc-152684-SH and Rag B shRNA (m) Lentiviral Particles: sc-152684-V as alternate gene silencing products.

For independent verification of Rag B (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-152684A, sc-152684B and sc-152684C.

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

Rag B siRNA (m) is recommended for the inhibition of Rag B 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

Rag B (2B8): sc-293349 is recommended as a control antibody for monitoring of Rag B 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 Rag B gene expression knockdown using RT-PCR Primer: Rag B (m)-PR: sc-152684-PR (20 μ l). 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.