

SNARK siRNA (h): sc-88608

BACKGROUND

The phosphorylation and dephosphorylation of proteins on serine and threonine residues is an essential means of regulating a broad range of cellular functions in eukaryotes, including cell division, homeostasis and apoptosis. A group of proteins that are intimately involved in this process are the serine/threonine (Ser/Thr) protein kinases. SNARK (SNF1/AMP kinase-related kinase), also known as NUA2 (NUAK family, SNF1-like kinase, 2), is a 628 amino acid protein that contains one protein kinase domain and belongs to the Ser/Thr protein kinase family. Using magnesium as a cofactor, SNARK catalyzes the ATP-dependent phosphorylation of target proteins and is involved in regulating cell tolerance to stress-induced glucose starvation. Additionally, SNARK is thought to induce cell-cell detachment and may protect cells from Fap-1-mediated apoptosis, possibly playing a role in the motility and invasiveness of tumor cells.

REFERENCES

1. Lefebvre, D.L., et al. 2001. Identification and characterization of a novel sucrose-non-fermenting protein kinase/AMP-activated protein kinase-related protein kinase, SNARK. *Biochem. J.* 355: 297-305.
2. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 608131. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
3. Suzuki, A., et al. 2003. Induction of cell-cell detachment during glucose starvation through F-Actin conversion by SNARK, the fourth member of the AMP-activated protein kinase catalytic subunit family. *Biochem. Biophys. Res. Commun.* 311: 156-161.

CHROMOSOMAL LOCATION

Genetic locus: NUA2 (human) mapping to 1q32.1.

PRODUCT

SNARK 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 SNARK shRNA Plasmid (h): sc-88608-SH and SNARK shRNA (h) Lentiviral Particles: sc-88608-V as alternate gene silencing products.

For independent verification of SNARK (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-88608A, sc-88608B and sc-88608C.

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

SNARK siRNA (h) is recommended for the inhibition of SNARK 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

SNARK (C-12): sc-374348 is recommended as a control antibody for monitoring of SNARK 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 SNARK gene expression knockdown using RT-PCR Primer: SNARK (h)-PR: sc-88608-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Bakula, D., et al. 2017. WIPI3 and WIPI4 β -propellers are scaffolds for LKB1-AMPK-TSC signalling circuits in the control of autophagy. *Nat. Commun.* 8: 15637.
2. Rosenthal, C.K. 2017. EGFR probes matrix stiffness. *Nat. Cell Biol.* 19: 600.

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.