

STOP siRNA (h): sc-63359

BACKGROUND

Microtubules in the cytoplasm of mammalian cells usually depolarize rapidly when exposed to cold temperature or to assembly-inhibiting drugs. Some cell types, however, contain sub-populations of microtubules called "cold-stable microtubules" that resist these depolymerizing conditions. This stabilization is due mainly to polymer association with a 952 amino acid neuronal protein designated STOP (stable tubule only polypeptide). The central region of STOP contains five tandem repeats of 46 amino acids. STOP also contains a SH3-binding motif near its N-terminus. It is present in the cell body and throughout the axon. The STOP protein action can be extreme, inducing resistance at temperatures as low as -80° C.

REFERENCES

1. Job, D., et al. 1987. High concentrations of STOP protein induce a microtubule super-stable state. *Biochem. Biophys. Res. Commun.* 148: 429-434.
2. Margolis, R.L., et al. 1987. Purification and assay of cold-stable microtubules and STOP protein. *Methods Enzymol.* 134: 160-170.
3. Pirollet, F., et al. 1989. Monoclonal antibody to microtubule-associated STOP protein: affinity purification of neuronal STOP activity and comparison of antigen with activity in neuronal and nonneuronal cell extracts. *Biochemistry* 28: 835-842.
4. Margolis, R.L., et al. 1991. Specific association of STOP protein with microtubules *in vitro* and with stable microtubules in mitotic spindles of cultured cells. *EMBO J.* 9: 4095-4102.
5. Bongiovanni, G., et al. 1994. Some common properties between a brain protein that is modified by posttranslational arginylation and the microtubule-associated STOP protein. *J. Neurochem.* 63: 2295-2299.
6. Denarier, E., et al. 1998. Nonneuronal isoforms of STOP protein are responsible for microtubule cold stability in mammalian fibroblasts. *Proc. Natl. Acad. Sci. USA* 95: 6055-6060.
7. Guillaud, L., et al. 1998. STOP proteins are responsible for the high degree of microtubule stabilization observed in neuronal cells. *J. Cell Biol.* 142: 167-179.
8. Galiano, M.R., et al. 2004. Astrocytes and oligodendrocytes express different STOP protein isoforms. *J. Neurosci. Res.* 78: 329-337.

CHROMOSOMAL LOCATION

Genetic locus: MAP6 (human) mapping to 11q13.5.

PRODUCT

STOP 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 STOP shRNA Plasmid (h): sc-63359-SH and STOP shRNA (h) Lentiviral Particles: sc-63359-V as alternate gene silencing products.

For independent verification of STOP (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-63359A, sc-63359B and sc-63359C.

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

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

STOP (175): sc-53513 is recommended as a control antibody for monitoring of STOP 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 STOP gene expression knockdown using RT-PCR Primer: STOP (h)-PR: sc-63359-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.

PROTOCOLS

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