

PGs2 siRNA (h): sc-76113

BACKGROUND

Polyglutamylation, polyglycylation and tyrosination are posttranslational modifications that tubulin undergoes in order to perform at maximal function. Polyglutamylation is evolutionarily conserved from protists to mammals and is involved in several microtubule functions such as axonemal beating, stability of centrioles, neuronal differentiation and mediating the interaction between tubulin and microtubule associated proteins. The neuronal tubulin polyglutamylase is a complex that contains a TTL (Tubulin tyrosine ligase-like) domain through which it catalyzes the ligation of glutamate to tubulins. The TTL domain contains ATP-grasp-like motifs that correspond to the ATP/Mg²⁺ binding site typical of enzymes with ATP-dependent carboxylate-amine/thiol ligase activity. PGs2 (Tubulin polyglutamylase complex subunit 2), also known as C18orf10, is a 300 amino acid cytoplasmic protein that participates in the neuronal Tubulin polyglutamylase complex, along with PGs1, PGs3, PGs4 and PGs5. There are two isoforms of PGs2 that are produced as a result of alternative splicing events.

REFERENCES

1. Boucher, D., et al. 1994. Polyglutamylation of tubulin as a progressive regulator of *in vitro* interactions between the microtubule-associated protein Tau and Tubulin. *Biochemistry* 33: 12471-12477.
2. Regnard, C., et al. 1996. Microtubules: functional polymorphisms of tubulin and associated proteins (structural and motor MAP's). *C. R. Seances Soc. Biol. Fil.* 190: 255-268.
3. Bonnet, C., et al. 2001. Differential binding regulation of microtubule-associated proteins MAP1A, MAP1B, and MAP2 by Tubulin polyglutamylase. *J. Biol. Chem.* 276: 12839-12848.
4. Regnard, C., et al. 2003. Characterisation of PGs1, a subunit of a protein complex co-purifying with Tubulin polyglutamylase. *J. Cell Sci.* 116: 4181-4190.

CHROMOSOMAL LOCATION

Genetic locus: TPGS2 (human) mapping to 18q12.2.

PRODUCT

PGs2 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 PGs2 shRNA Plasmid (h): sc-76113-SH and PGs2 shRNA (h) Lentiviral Particles: sc-76113-V as alternate gene silencing products.

For independent verification of PGs2 (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-76113A, sc-76113B and sc-76113C.

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

PGs2 siRNA (h) is recommended for the inhibition of PGs2 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

PGs2 (D-9): sc-514306 is recommended as a control antibody for monitoring of PGs2 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 PGs2 gene expression knockdown using RT-PCR Primer: PGs2 (h)-PR: sc-76113-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.