

PGs5 siRNA (m): sc-152197

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. PGs5 (Tubulin polyglutamylase complex subunit 5), also known as Nicotin-1, is a 213 amino acid nuclear protein that participates in the neuronal Tubulin polyglutamylase complex, along with PGs1, PGs2, PGs3 and PGs4. PGs5 is highly expressed in liver, brain, testis and kidney, with weaker expression found in colon, small intestine, leukocytes and spleen. There are two isoforms of PGs5 that are produced as a result of alternative splicing events.

REFERENCES

1. Boucher, D., Larcher, J.C., Gros, F. and Denoulet, P. 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., Audebert, S., Boucher, D., Larcher, J.C., Edde, B. and Denoulet, P. 1996. Microtubules: functional polymorphisms of Tubulin and associated proteins (structural and motor MAP's). *C. R. Seances Soc. Biol. Fil.* 190: 255-268.
3. Backofen, B. and Leeb, T. 2002. Genomic organization of the murine aminomethyltransferase gene (Amt). *DNA Seq.* 13: 179-183.
4. Backofen, B., Jacob, R., Serth, K., Gossler, A., Naim, H.Y. and Leeb, T. 2002. Cloning and characterization of the mammalian-specific nicotin 1 gene (NICN1) encoding a nuclear 24 kDa protein. *Eur. J. Biochem.* 269: 5240-5245.
5. Regnard, C., Fesquet, D., Janke, C., Boucher, D., Desbruyeres, E., Koulakoff, A., Insina, C., Travo, P. and Edde, B. 2003. Characterisation of PGs1, a subunit of a protein complex co-purifying with Tubulin polyglutamylase. *J. Cell Sci.* 116: 4181-4190.
6. Janke, C., Rogowski, K., Wloga, D., Regnard, C., Kajava, A.V., Strub, J.M., Temurak, N., van Dijk, J., Boucher, D., van Dorsselaer, A., Suryavanshi, S., Gaertig, J. and Edde, B. 2005. Tubulin polyglutamylase enzymes are members of the TTL domain protein family. *Science* 308: 1758-1762.
7. Chen, L.L., DeCervo, J.N. and Carmichael, G.G. 2008. Alu element-mediated gene silencing. *EMBO J.* 27: 1694-1705.
8. Janke, C., Rogowski, K. and van Dijk, J. 2008. Polyglutamylation: a fine-regulator of protein function? "Protein modifications: beyond the usual suspects" review series. *EMBO Rep.* 9: 636-641.
9. Fukushima, N., Furuta, D., Hidaka, Y., Moriyama, R. and Tsujiuchi, T. 2009. Post-translational modifications of Tubulin in the nervous system. *J. Neurochem.* 109: 683-693.

CHROMOSOMAL LOCATION

Genetic locus: Nicn1 (mouse) mapping to 9 F2.

PRODUCT

PGs5 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 PGs5 shRNA Plasmid (m): sc-152197-SH and PGs5 shRNA (m) Lentiviral Particles: sc-152197-V as alternate gene silencing products.

For independent verification of PGs5 (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-152197A, sc-152197B and sc-152197C.

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

PGs5 siRNA (m) is recommended for the inhibition of PGs5 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PGs5 gene expression knockdown using RT-PCR Primer: PGs5 (m)-PR: sc-152197-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.