

PAPD1 siRNA (h): sc-90573

BACKGROUND

PAPD1 (PAP-associated domain-containing protein 1), also known as MTPAP (mitochondrial poly(A) RNA polymerase), is a 582 amino acid cytoplasmic protein that contains one PAP-associated domain and belongs to the DNA polymerase type-B-like family. PAPD1 exists as two alternatively spliced isoforms and forms a homodimer. As a polymerase that creates the 3' poly(A) tail of mitochondrial transcripts, PAPD1 can use all four nucleotides, but has higher activity with ATP and UTP. PAPD1 also plays a role in replication-dependent histone mRNA degradation and may be involved in the terminal uridylation of mature histone mRNAs before their degradation is initiated. While it is ubiquitously expressed, PAPD1 exhibits stronger expression in tissues with high energy requirements, such as heart, brain and skeletal muscle. Defects in PAPD1 are the cause of spastic ataxia autosomal recessive type 4 (SPAX4), a slowly progressive neurodegenerative disease characterized by cerebellar ataxia, spastic paraparesis, dysarthria and optic atrophy.

REFERENCES

- Berger, P., Young, P. and Suter, U. 2002. Molecular cell biology of Charcot-Marie-Tooth disease. *Neurogenetics* 4: 1-15.
- Tomecki, R., Dmochowska, A., Gewartowski, K., Dziembowski, A. and Stepień, P.P. 2004. Identification of a novel human nuclear-encoded mitochondrial poly(A) polymerase. *Nucleic Acids Res.* 32: 6001-6014.
- Nagaike, T., Suzuki, T., Katoh, T. and Ueda, T. 2005. Human mitochondrial mRNAs are stabilized with polyadenylation regulated by mitochondria-specific poly(A) polymerase and polynucleotide phosphorylase. *J. Biol. Chem.* 280: 19721-19727.
- Xiao, Q., Wu, X.L., Michal, J.J., Reeves, J.J., Busboom, J.R., Thorgaard, G.H. and Jiang, Z. 2006. A novel nuclear-encoded mitochondrial poly(A) polymerase PAPD1 is a potential candidate gene for the extreme obesity related phenotypes in mammals. *Int. J. Biol. Sci.* 2: 171-178.
- Crosby, A.H., Patel, H., Chioza, B.A., Proukakis, C., Gurtz, K., Patton, M.A., Sharifi, R., Harlalka, G., Simpson, M.A., Dick, K., Reed, J.A., Al-Memar, A., Chrzanowska-Lightowlers, Z.M., Cross, H.E. and Lightowlers, R.N. 2010. Defective mitochondrial mRNA maturation is associated with spastic ataxia. *Am. J. Hum. Genet.* 87: 655-660.
- Laugel, V., Dalloz, C., Durand, M., Sauvanaud, F., Kristensen, U., Vincent, M.C., Pasquier, L., Odent, S., Cormier-Daire, V., Gener, B., Tobias, E.S., Tolmie, J.L., Martin-Coignard, D., Drouin-Garraud, V., Heron, D., et al. 2010. Mutation update for the CSB/ERCC6 and CSA/ERCC8 genes involved in Cockayne syndrome. *Hum. Mutat.* 31: 113-126.
- Online Mendelian Inheritance in Man, OMIM[™]. 2010. Johns Hopkins University, Baltimore, MD. MIM Number: 613669. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
- Bai, Y., Srivastava, S.K., Chang, J.H., Manley, J.L. and Tong, L. 2011. Structural basis for dimerization and activity of human PAPD1, a non-canonical poly(A) polymerase. *Mol. Cell* 41: 311-320.

CHROMOSOMAL LOCATION

Genetic locus: MTPAP (human) mapping to 10p11.23.

PRODUCT

PAPD1 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 PAPD1 shRNA Plasmid (h): sc-90573-SH and PAPD1 shRNA (h) Lentiviral Particles: sc-90573-V as alternate gene silencing products.

For independent verification of PAPD1 (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-90573A, sc-90573B and sc-90573C.

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

PAPD1 siRNA (h) is recommended for the inhibition of PAPD1 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PAPD1 gene expression knockdown using RT-PCR Primer: PAPD1 (h)-PR: sc-90573-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.