

PAP- γ siRNA (m): sc-155928

BACKGROUND

Polyadenylation of the 3' ends of eukaryotic mRNAs is a key event that takes place in the nucleus during maturation of mRNA. The reaction occurs in two distinct steps: endoribonucleolytic cleavage of the pre-mRNA at the poly(A) site, followed by synthesis of the poly(A) tail at the 3' end of the upstream cleavage product. PAP- γ (poly(A) polymerase γ), also known as PAPOLG, neo-poly(A) polymerase, SRP RNA 3'-adenylating enzyme, PAP2 or PAPG, is a 736 amino acid nuclear protein that belongs to the poly(A) polymerase family, which catalyzes extension of the 3' end of DNA or RNA and is required for the adenosine addition reaction during polyadenylation. Predominantly expressed in testis but found at low levels in other tissues, PAP- γ is overexpressed in various tumors and is encoded by a gene that maps to human chromosome 2p16.1.

REFERENCES

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3. Perumal, K., Sinha, K., Henning, D. and Reddy, R. 2001. Purification, characterization, and cloning of the cDNA of human signal recognition particle RNA 3'-adenylating enzyme. *J. Biol. Chem.* 276: 21791-21796.
4. Kyriakopoulou, C.B., Nordvang, H. and Virtanen, A. 2001. A novel nuclear human poly(A) polymerase (PAP), PAP- γ . *J. Biol. Chem.* 276: 33504-33511.
5. Topalian, S.L., Kaneko, S., Gonzales, M.I., Bond, G.L., Ward, Y. and Manley, J.L. 2001. Identification and functional characterization of neo-poly(A) polymerase, an RNA processing enzyme overexpressed in human tumors. *Mol. Cell. Biol.* 21: 5614-5623.
6. Lee, Y.S., Johnson, K.A., Molineux, I.J. and Yin, Y.W. 2010. A single mutation in human mitochondrial DNA polymerase Pol γ A affects both polymerization and proofreading activities of only the holoenzyme. *J. Biol. Chem.* 285: 28105-28116.

CHROMOSOMAL LOCATION

Genetic locus: Papolg (mouse) mapping to 11 A3.2.

PRODUCT

PAP- γ 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 PAP- γ shRNA Plasmid (m): sc-155928-SH and PAP- γ shRNA (m) Lentiviral Particles: sc-155928-V as alternate gene silencing products.

For independent verification of PAP- γ (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-155928A, sc-155928B and sc-155928C.

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

PAP- γ siRNA (m) is recommended for the inhibition of PAP- γ 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.

GENE EXPRESSION MONITORING

PAP- $\alpha/\beta/\gamma$ (D-1): sc-365607 is recommended as a control antibody for monitoring of PAP- γ 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 λ BP-HRP: sc-516132 or m-IgG λ BP-HRP (Cruz Marker): sc-516132-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 λ BP-FITC: sc-516185 or m-IgG λ BP-PE: sc-516186 (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 PAP- γ gene expression knockdown using RT-PCR Primer: PAP- γ (m)-PR: sc-155928-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.