

PAP- β siRNA (m): sc-40807

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 includes endoribonucleolytic cleavage of the pre-mRNA at the poly(A) site that leads to synthesis of the poly(A) tail at the 3' end of the upstream cleavage product. The poly(A) polymerase (PAP) is required. The adenosine addition reaction depends on poly(A) polymerase (PAP) activity. The testis expresses PAP- β (TPAP) in the cytoplasm of spermatogenic cells. The adenosine addition function of PAP- β plays a critical role in male germ cell production. PAP- β -deficient transgenic mice display impaired expression of haploid-specific genes that are necessary for spermatogenesis. The intronless gene encoding human PAP- β maps to chromosome 7p22.3.

REFERENCES

1. Christofori, G. and Keller, W. 1989. Poly(A) polymerase purified from HeLa cell nuclear extract is required for both cleavage and polyadenylation of pre-mRNA *in vitro*. *Mol. Cell. Biol.* 9: 193-203.
2. Lee, Y.J., Lee, Y. and Chung, J.H. 2000. An intronless gene encoding a poly(A) polymerase is specifically expressed in testis. *FEBS Lett.* 487: 287-292.
3. Kashiwabara, S., Zhuang, T., Yamagata, K., Noguchi, J., Fukamizu, A. and Baba, T. 2000. Identification of a novel isoform of poly(A) polymerase, TPAP, specifically present in the cytoplasm of spermatogenic cells. *Dev. Biol.* 228: 106-115.
4. Kashiwabara, S., Noguchi, J., Zhuang, T., Ohmura, K., Honda, A., Sugiura, S., Miyamoto, K., Takahashi, S., Inoue, K., Ogura, A. and Baba, T. 2002. Regulation of spermatogenesis by testis-specific, cytoplasmic poly(A) polymerase TPAP. *Science* 298: 1999-2002.
5. LocusLink Report (LocusID: 56903). <http://www.ncbi.nlm.nih.gov/LocusLink>

CHROMOSOMAL LOCATION

Genetic locus: Papolb (mouse) mapping to 5 G2.

PRODUCT

PAP- β 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- β shRNA Plasmid (m): sc-40807-SH and PAP- β shRNA (m) Lentiviral Particles: sc-40807-V as alternate gene silencing products.

For independent verification of PAP- β (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-40807A, sc-40807B and sc-40807C.

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

PAP- β siRNA (m) is recommended for the inhibition of PAP- β 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- β 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- β gene expression knockdown using RT-PCR Primer: PAP- β (m)-PR: sc-40807-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.