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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

SANTA CRUZ BIOTECHNOLOGY, INC.

PAP-α siRNA (m): sc-152010

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-RNA at the poly(A) site, followed by synthesis of the poly(A) tail at the 3' end of the upstream cleavage product. The poly(A) polymerase (PAP) is required for the adenosine addition reaction. Western blot analysis reveals three PAPs, namely PAP-α, PAP-β and PAP-γ, demonstrating different molecular masses in HeLa cell extracts. The amino-terminal region of PAP is required for nonspecific polymerase activity, while both the amino and carboxy termini are required for specific polymerase activity. Additionally, PAP contains a functional ribonucleoprotein-type RNA binding domain (RBD) that is responsible for primer binding.

REFERENCES

1. Weichs an der Glon, C., et al. 1993. Tat-dependent occlusion of the HIV poly(A) site. EMBO J. 12: 2119-2128.
2. Thuresson, A.C., et al. 1994. Multiple forms of poly(A) polymerases in human cells. Proc. Natl. Acad. Sci. USA 91: 979-983.
3. Pendurthi, U.R., et al. 1997. Binding of factor VIIa to tissue factor induces alterations in gene expression in human fibroblast cells: up-regulation of poly(A) polymerase. Proc. Natl. Acad. Sci. USA 94: 12598-12603.
4. Yamauchi, T., et al. 1999. Assignment of the human poly(A) polymerase (PAP) gene to chromosome 14q32.1-q32.2 and isolation of a polymorphic CA repeat sequence. J. Hum. Genet. 44: 253-255.
5. Mouland, A.J., et al. 2002. Hypophosphorylation of poly(A) polymerase and increased polyadenylation activity are associated with human immunodeficiency virus type 1 Vpr expression. Virology 292: 321-330.
6. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 605553. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
7. Kim, H., et al. 2003. Regulation of poly(A) polymerase by 14-3-3ε. EMBO J. 22: 5208-5219.
8. Kaufmann, I., et al. 2004. Human Fip1 is a subunit of CPSF that binds to U-rich RNA elements and stimulates poly(A) polymerase. EMBO J. 23: 616-626.

CHROMOSOMAL LOCATION

Genetic locus: Papola (mouse) mapping to 12 E.

PRODUCT

PAP-α 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PAP-α shRNA Plasmid (m): sc-152010-SH and PAP-α shRNA (m) Lentiviral Particles: sc-152010-V as alternate gene silencing products.

For independent verification of PAP-α (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-152010A, sc-152010B and sc-152010C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

PAP-α siRNA (m) is recommended for the inhibition of PAP-α 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 µM in 66 µl. 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-α/β/γ (D-1): sc-365607 is recommended as a control antibody for monitoring of PAP-α 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-IgGλ BP-HRP: sc-516132 or m-IgGλ 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-IgGλ BP-FITC: sc-516185 or m-IgGλ 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-α gene expression knockdown using RT-PCR Primer: PAP-α (m)-PR: sc-152010-PR (20 µl). 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.