

PAP- $\alpha/\beta/\gamma$ (T-20): sc-20188

BACKGROUND

Polyadenylation of the 3-prime 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-prime 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., Ashe, M., Eggermont, J. and Proudfoot, N.J. 1993. Tat-dependent occlusion of the HIV poly(A) site. *EMBO J.* 12: 2119-2128.
2. Thureson, A.C., Aström, J., Aström, A., Grönvik, K.O. and Virtanen, A. 1994. Multiple forms of poly(A) polymerases in human cells. *Proc. Natl. Acad. Sci. USA* 91: 979-983.
3. Pendurthi, U.R., Alok, D. and Rao, L.V. 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., Sugimoto, J., Hatakeyama, T., Asakawa, S., Shimizu, N. and Isobe, M. 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. Moulard, A.J., Coady, M., Yao, X.J. and Cohen, E.A. 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., Lee, J.H. and Lee, Y. 2003. Regulation of poly(A) polymerase by 14-3-3 ϵ . *EMBO J.* 22: 5208-5219.
8. Kaufmann, I., Martin, G., Friedlein, A., Langen, H. and Keller, W. 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.
9. Meinke, G., Ezeokonkwo, C., Balbo, P., Stafford, W., Moore, C. and Bohm, A. 2008. Structure of yeast poly(A) polymerase in complex with a peptide from Fip1, an intrinsically disordered protein. *Biochemistry* 47: 6859-6869.

SOURCE

PAP- $\alpha/\beta/\gamma$ (T-20) is an affinity purified goat polyclonal antibody raised against a peptide mapping within an internal region of PAP of human origin.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PRODUCT

Each vial contains 200 μ g IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-20188 P, (100 μ g peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% BSA).

APPLICATIONS

PAP- $\alpha/\beta/\gamma$ (T-20) is recommended for detection of PAP- α , PAP- β and PAP- γ of mouse, rat and human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

PAP- $\alpha/\beta/\gamma$ (T-20) is also recommended for detection of PAP- α , PAP- β and PAP- γ in additional species, including equine, canine, bovine, porcine and avian.

Molecular Weight of PAP- $\alpha/\beta/\gamma$: 64 kDa.

Positive Controls: Rat testis extract: sc-2400 or mouse testis extract: sc-2405.

RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker[™] compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker[™] Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz[™] Mounting Medium: sc-24941.

STORAGE

Store at 4° C, ****DO NOT FREEZE****. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.



Try **PAP- $\alpha/\beta/\gamma$ (D-1): sc-365607**, our highly recommended monoclonal alternative to PAP- $\alpha/\beta/\gamma$ (T-20).