SANTA CRUZ BIOTECHNOLOGY, INC.

PAP (H-300): sc-32915

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

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, 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. The gene which encodes PAP maps to human chromosome 14q32.2.

REFERENCES

1. Christofori, G. et al. 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. Thuresson, A.C., et al. 1994. Multiple forms of poly(A) polymerases in human cells. Proc. Natl. Acad. Sci. USA 91: 979-983.
3. Raabe, T., et al. 1994. Poly(A) polymerase contains multiple functional domains. Mol. Cell. Biol. 14: 2946-2957.
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: 25325-25325.

SOURCE

PAP (H-300) is a rabbit polyclonal antibody raised against amino acids 1-300 mapping at the N-terminus of PAP-α of human origin.

PRODUCT

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

RESEARCH USE

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

APPLICATIONS

PAP (H-300) is recommended for detection of all PAP isoforms of mouse, rat and human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], 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 (H-300) is also recommended for detection of all PAP isoforms in additional species, including bovine, porcine and avian.

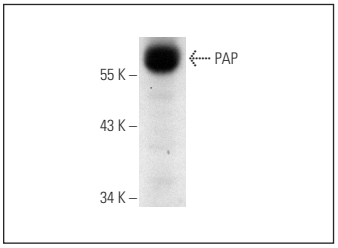
Molecular Weight of PAP: 64 kDa.

Positive Controls: PAP-α (h): 293 Lysate: sc-110765, rat testis extract: sc-2400 or mouse testis extract: sc-2405.

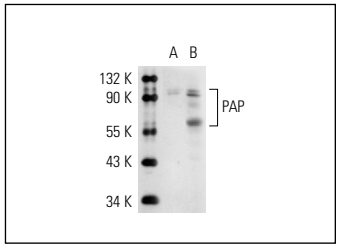
RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

DATA



PAP (H-300): sc-32915. Western blot analysis of PAP expression in mouse testis tissue extract.



PAP (H-300): sc-32915. Western blot analysis of PAP expression in non-transfected: sc-110760 (**A**) and human PAP transfected: sc-110765 (**B**) 293 whole cell lysates.

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.