ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Proliferating Cell Nuclear Antigen antibody

☐ Cat # PCNA11-A	Rabbit anti-Human PCNA antibody	**SIZE**: 100 µg
☐ Cat # PCNA11-C	Recombinant Human PCNA protein control for Western blotting	**SIZE**: 100 µl

PCNA (Proliferating cell nuclear antigen) PCNA is a DNA clamp that acts as a processivity factor for DNA polymerase δ in eukaryotic cells and is essential for replication. PCNA is a homotrimer and achieves its processivity by encircling the DNA, where it acts as a scaffold to recruit proteins involved in DNA replication, DNA repair, chromatin remodeling and epigenetics. The sequence of PCNA is well conserved between plants and animals, indicating a strong selective pressure for structure conservation, and suggesting that this type of DNA replication mechanism is conserved throughout eukaryotes. In Saccharomyces cerevisiae (Baker's yeast), POL30, is associated with polymerase III, the yeast analog of polymerase delta. PCNA is also able to interact with a wide variety of cell cycle proteins and serves to coordinate several proteins participating in DNA processes, such as apoptosis.

Source of Antigen or Antibodies

Uniprot: P12004

Host: Rabbit

Clonality: Polyclonal

Immunogen: Full length recombinant SF9 expressed Human PCNA

Purification: Ammonium sulfate followed by protein affinity purification

Species Reactivity: Human

Cross reactivity: PCNA is highly conserved across various species. We recommend performing a BLAST with Human PCNA to determine if the antibody will react with your target species. Generally, over 80% homology may exhibit reactivity.

Subcellular Location: Nuclear

Recommended Secondary Antibody: Goat anti-Rabbit IgG-HRP (**ADI cat#20320)**

Negative Control: Non-immune Rabbit IgG (**ADI cat# 20009-1**).

PCNA11-C: Contains a recombinant SF9 expressed full length human PCNA protein at a concentration of 1 ng/µl in Laemmli buffer (62.5 mM Tris-HCL, pH 6.8, 2% SDS, 10% glycerol, 5% BME, and 0.002% bromphenol blue). Heat for 5 minutes at 95°C then load 1-5 µl. Store at -20°C in suitable size aliquots, do not expose to multiple free/thaw cycles. **Note:** Due to the addition of tags, the protein appears slightly larger than native PCNA.

Form & Storage of Antibodies

☐ **Affinity pure IgG Solution**
Concentration: 0.5 mg/ml Volume: 200 µl
Supplied in PBS, pH 7.4 + 0.1% BSA
The antibody can be made available carrier free or conjugated to HRP, Biotin, or FITC on request

☐ **Lyophilized powder**
Reconstitute powder in 200 µl distilled water to 0.5 mg/ml

Storage:

Short-term: 4°C for 1 month

Long-term: at –20°C or below in suitable aliquots after reconstitution for 1 year. Do not expose to multiple free/thaw cycles or store working, diluted solutions. Glycerol may be added to a final concentration of 50% and antibodies can be stored un-aliquoted at –20°C.

Recommended Usage

ELISA: Assay dependent concentration. Typically, between 0.1-2.0 µg/ml for capture/detection antibodies.

Western Blotting: 0.5-1.0 µg/ml
Predicted band size: 28.8 kDa
Observed band size: 37 kDa.

IHC-P: 1-10 µg/ml. No staining was observed with pH 6 sodium citrate antigen retrieval buffer. We recommend Tris-EDTA pH 9 antigen retrieval buffer.

The above concentrations are a *suggestion*, user's must optimize their assay based on their own conditions. The antibody may work in other applications such as Immunocytochemistry or IP. These methods have not been tested by ADI.

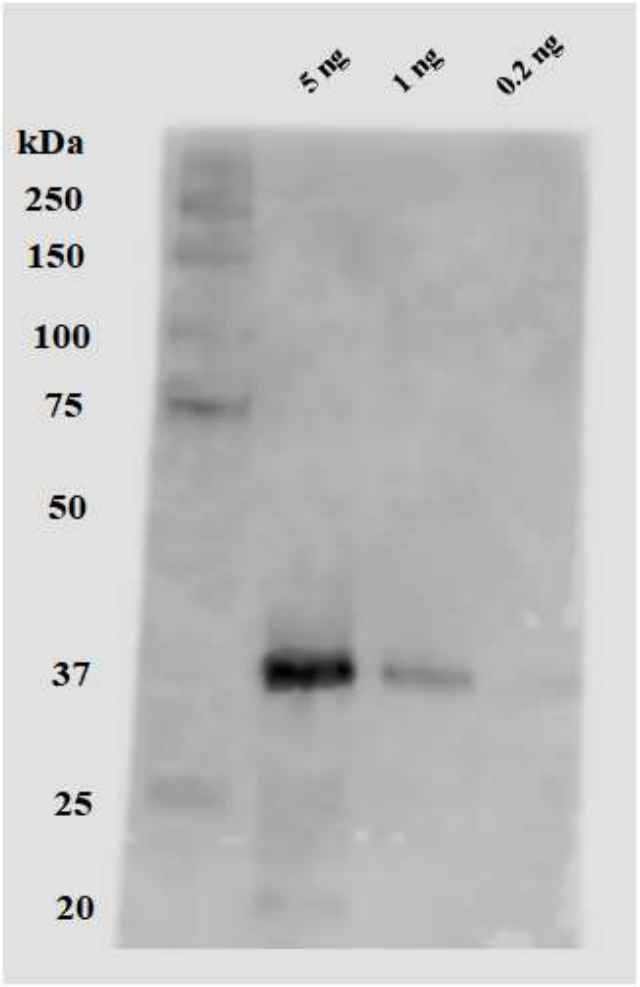
This product is for In vitro research use only.

Related materials available from ADI

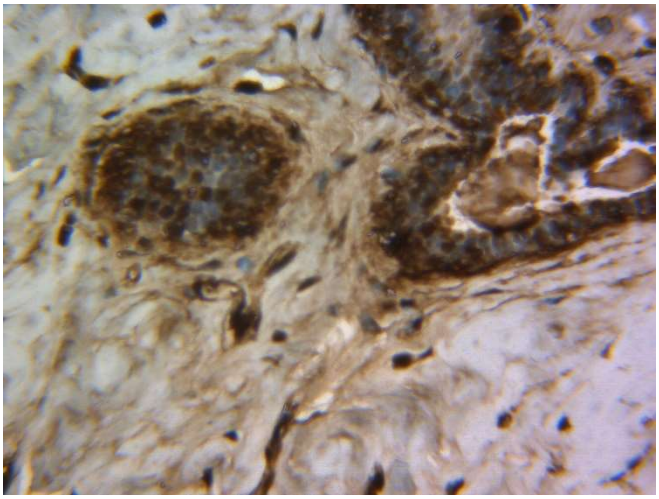
Catalog#	Description
BCL11-A	Rabbit Anti-Human BCL-2 antibody
BCL21-A	Rabbit Anti-Mouse BCL-2 antibody
BCL2-C	Recombinant BCL-2 control for Western blotting
HP5311-A	Rabbit anti-Human P53 antibody
HP5311-C	Recombinant Human P53 protein control for Western blotting
CASP3-A	Rabbit anti-Mouse Capase 3 antibody
AKT1-A	Rabbit anti-Human AKT1 antibody
AKT1-C	Recombinant AKT1 control for Western blotting

PCNA11-A 192801IA

Alpha Diagnostic Intl Inc., 6203 Woodlake Center Dr, San Antonio, TX 78244, USA; **Email**: service@4adi.com
☎ (800) 786-5777; (210) 561-9515; **Fax**: (210) 561-9544; **Web Site**: www.4adi.com



Western blotting: 5, 1, and 0.2 ng of recombinant PCNA was heated for 5 minutes at 95ºC then electrophoretically separated on a 10% SDS-PAGE gel. The gel was run at 100V for ~1 hour and 30 minutes then transferred to a 0.2 µm nitrocellulose membrane using the 'Mixed MW' settings on a Transblot Turbo (Biorad). The blot was blocked for 1 hour at room temperature with 1% Casein. **PCNA11-A** was diluted with TBST+0.1% BSA to 1 ug/ml and incubated overnight at 4ºC. The blot was washed with TBS-T 3 times for 5 minutes each. Goat anti-rabbit IgG HRP (**ADI cat#20320**) was diluted in TBST+0.1% BSA at a 1:10,000 dilution (50 ng/ml) then incubated for 1 hour at room temperature. The blot was washed 3 times with TBS-T for 5 minutes each. The blot was then incubated with ADI Femto ECL substrate (**ADI cat#80210**) for 5 minutes and imaged on a CCD imaging system (C-DiGit, LI-COR).



Immunohistochemistry: Human adenocarcinoma slide was heated for 20 minutes at 60ºC then deparrafinized. Antigen retrieval was performed for 10 minutes at 95ºC in a microwave using Tris-EDTA pH 9 antigen retrieval buffer. The slide was then cooled for 20 minutes at room temperature before being blocked for 30 minutes with 2.5% normal goat serum. **PCNA11-A** was diluted to 5 µg/ml in TBST+0.1% BSA and incubated overnight at 4ºC. The slide was then washed twice and incubated with 3% hydrogen peroxide for 10 minutes to quench endogenous peroxidase. The slide was washed then incubated with Goat anti-Rabbit IgG HRP polymer detection reagent for 30 minutes at room temperature. The slide was washed twice, incubated with DAB for 3 minutes, washed with distilled water, then counterstained for 1 minute with Gil's II Hematoxylin before being cover-slipped.

ng/ml	50	10	2	0.4	0.08	0.016	0
OD_{450}	2.971	3.001	2.857	1.078	0.277	0.049	0

ELISA: The activity of the antibody was assessed in an indirect format. Recombinant PCNA was coated on a 96 well microwell plate. 100 µl of purified PCNA antibody was added at a concentration of 50 ng/ml and diluted 5-fold. After 1 hour, the wells were washed and Goat anti-rabbit IgG HRP was added for 30 minutes. The wells were washed and TMB substrate was added for 15 minutes. The reaction was stopped with 1% sulfuric acid and read at A_{450} nm.

LOD: The antibody shows detectable activity below a concentration of 20 pg/ml in an indirect format.