

# Anti-PCNA Rabbit Monoclonal Antibody

Catalog # ABO13291

## Specification

## **Anti-PCNA Rabbit Monoclonal Antibody - Product Information**

Application Primary Accession Host Isotype Reactivity Clonality Format Description WB, IHC, IF, ICC, IP, FC <u>P12004</u> Rabbit Rabbit IgG Rat, Human, Mouse Monoclonal Liquid

Anti-PCNA Rabbit Monoclonal Antibody . Tested in WB, IHC, ICC/IF, IP, Flow Cytometry applications. This antibody reacts with Human, Mouse, Rat.

## Anti-PCNA Rabbit Monoclonal Antibody - Additional Information

Gene ID 5111

Other Names Proliferating cell nuclear antigen, PCNA, Cyclin, PCNA

Calculated MW 28769 MW KDa

Application Details WB 1:3000-1:10000<br>IHC 1:50-1:200<br>ICC/IF 1:100-1:500<br>IP 1:50-1:100<br>FC 1:200-1:500

Subcellular Localization

Nucleus. Colocalizes with CREBBP, EP300 and POLD1 to sites of DNA damage (PubMed:24939902). Forms nuclear foci representing sites of ongoing DNA replication and vary in morphology and number during S phase. Together with APEX2, is redistributed in discrete nuclear foci in presence of oxidative DNA damaging agents..

**Contents** Rabbit IgG in phosphate buffered saline, pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol, 0.4-0.5mg/ml BSA.

Immunogen A synthesized peptide derived from human PCNA

Purification Affinity-chromatography

Storage

Store at -20°C for one year. For short term storage and frequent use, store at 4°C for up to one month. Avoid repeated



freeze-thaw cycles.

## Anti-PCNA Rabbit Monoclonal Antibody - Protein Information

#### Name PCNA

#### **Function**

Auxiliary protein of DNA polymerase delta and epsilon, is involved in the control of eukaryotic DNA replication by increasing the polymerase's processibility during elongation of the leading strand (PubMed:<a href="http://www.uniprot.org/citations/35585232" target="\_blank">35585232</a>). Induces a robust stimulatory effect on the 3'-5' exonuclease and 3'-phosphodiesterase, but not apurinic-apyrimidinic (AP) endonuclease, APEX2 activities. Has to be loaded onto DNA in order to be able to stimulate APEX2. Plays a key role in DNA damage response (DDR) by being conveniently positioned at the replication fork to coordinate DNA replication with DNA repair and DNA damage tolerance pathways (PubMed:<a href="http://www.uniprot.org/citations/24939902" target="\_blank">24939902</a>). Acts as a loading platform to recruit DDR proteins that allow completion of DNA replication after DNA damage and promote postreplication repair: Monoubiquitinated PCNA leads to recruitment of translesion (TLS) polymerases, while 'Lys-63'-linked polyubiquitination of PCNA is involved in error-free pathway and employs recombination mechanisms to synthesize across the lesion (PubMed:<a href="http://www.uniprot.org/citations/24695737" target=" blank">24695737</a>).

#### **Cellular Location**

Nucleus. Note=Colocalizes with CREBBP, EP300 and POLD1 to sites of DNA damage (PubMed:24939902). Forms nuclear foci representing sites of ongoing DNA replication and vary in morphology and number during S phase (PubMed:15543136). Co-localizes with SMARCA5/SNF2H and BAZ1B/WSTF at replication foci during S phase (PubMed:15543136). Together with APEX2, is redistributed in discrete nuclear foci in presence of oxidative DNA damaging agents

## **Anti-PCNA Rabbit Monoclonal Antibody - Protocols**

Provided below are standard protocols that you may find useful for product applications.

- <u>Western Blot</u>
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- <u>Cell Culture</u>

Anti-PCNA Rabbit Monoclonal Antibody - Images





Figure 1. Western blot analysis of PCNA using anti-PCNA antibody (M00125-1).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: human Hela whole cell lysates,

Lane 2: human HepG2 whole cell lysates,

Lane 3: human U-937 whole cell lysates,

Lane 4: human 293T whole cell lysates,

Lane 5: mouse spleen tissue lysates,

Lane 6: rat RAW264.7 tissue lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-PCNA antigen affinity purified monoclonal antibody (Catalog # M00125-1) at 1:3000 overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for PCNA at approximately 36 kDa. The expected band size for PCNA is at 29 kDa.



Immunoprecipitate (IP) analysis using the Antibody at 1:50 dilution. (wb at 1:3K dilution)





Figure 3. IF analysis of PCNA using anti-PCNA antibody (M00125-1) and anti-Beta Tubulin antibody (M01857-3).

PCNA was detected in immunocytochemical section of U2OS cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated at 1:100 with rabbit anti-PCNA Antibody (M00125-1) and mouse anti-Beta Tubulin antibody (M01857-3) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) and Dylight550-conjugated Anti- mouse IgG Secondary Antibody (red)(Catalog#BA1133) were used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. Visualize using a fluorescence microscope and filter sets appropriate for the label used.