abcam

Product datasheet

Anti-PCNA antibody [EPR3821] ab92552

Recombinant RabMAb

★★★★★ 12 Abreviews 354 References 17 Images

Overview

Product name	Anti-PCNA antibody [EPR3821]
Description	Rabbit monoclonal [EPR3821] to PCNA
Host species	Rabbit
Tested applications	**Suitable for:** WB, IP, Flow Cyt (Intra), IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Mouse spleen lysate. HeLa, NIH/3T3, PC-12, HepG2, HEK-293, HEK-293T and A431 cell lysates. IHC-P: Human ovarian carcinoma, urinary bladder carcinoma, normal colon, breast carcinoma and cervical carcinoma tissue. Rat liver tissue. Mouse testis tissue. ICC/IF: A431 and HeLa cells. IP: HeLa cell lysate. Flow Cyt (intra): HeLa cells.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3821

Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab92552 in the following tested applications.

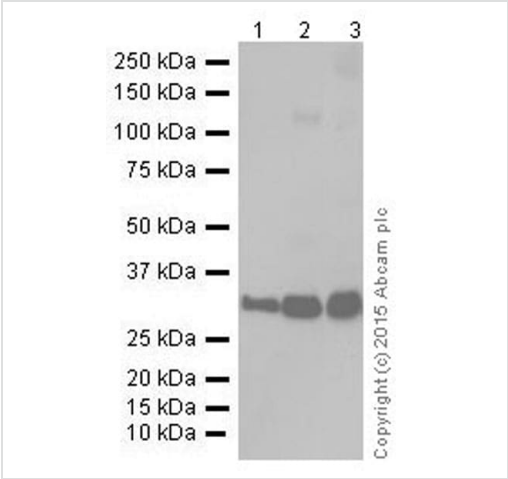
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★☆ (5)	1/1000 - 1/10000. Predicted molecular weight: 29 kDa.
IP		1/20 - 1/50.
Flow Cyt (Intra)		1/40. For unpurified, use 1/1000. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P	★★★★★ (5)	1/100 - 1/1000. Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**. The use of an HRP/AP polymerized antibody is recommended for a secondary antibody.
ICC/IF	★★★★☆ (1)	1/100 - 1/250. Use with methanol fixed samples.

Target

Function	This protein is an auxiliary protein of DNA polymerase delta and is involved in the control of eukaryotic DNA replication by increasing the polymerase's processibility during elongation of the leading strand. 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.
Sequence similarities	Belongs to the PCNA family.
Post-translational modifications	Upon methyl methanesulfonate-induced DNA damage, mono-ubiquitinated by the UBE2B-RAD18 complex on Lys-164. This induces non-canonical polyubiquitination on Lys-164 through 'Lys-63' linkage of ubiquitin moieties by the E2 complex UBE2N-UBE2V2 and the E3 ligases, HLTF, RNF8 and SHPRH, which is required for DNA repair. 'Lys-63' polyubiquitination prevents genomic instability on DNA damage. Monoubiquitination at Lys-164 also takes place in undamaged proliferating cells, and is mediated by the DCX(DTL) complex, leading to enhance PCNA-dependent translesion DNA synthesis. Acetylated in response to UV irradiation. Acetylation disrupts interaction with NUDT15 and promotes degradation.
Cellular localization	Nucleus. 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.

Images



Western blot - Anti-PCNA antibody [EPR3821] (ab92552)

All lanes : Anti-PCNA antibody [EPR3821] (ab92552) at 1/1000 dilution (purified)

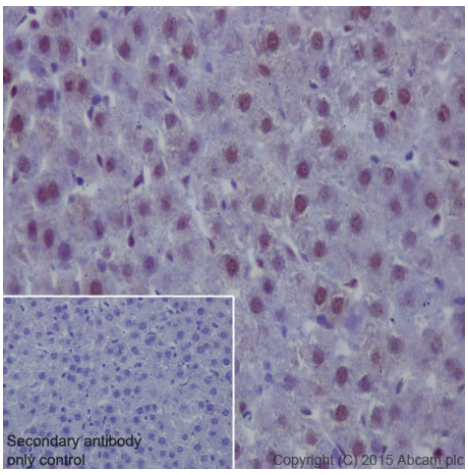
Lane 1 : Mouse spleen lysate
Lane 2 : NIH/3T3 (Mouse embryo fibroblast cell line) lysate
Lane 3 : PC-12 (Rat adrenal gland pheochromocytoma cell line) lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit IgG (H+L) at 1/20000 dilution

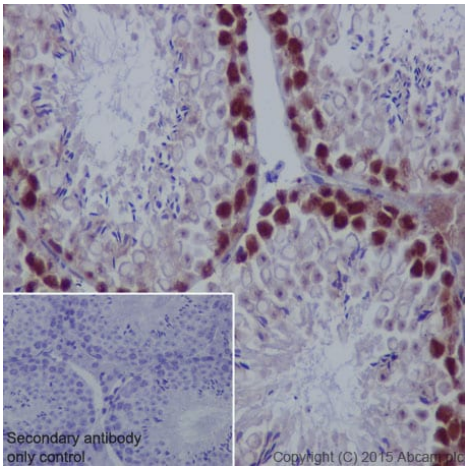
Predicted band size: 29 kDa
Observed band size: 29 kDa

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST



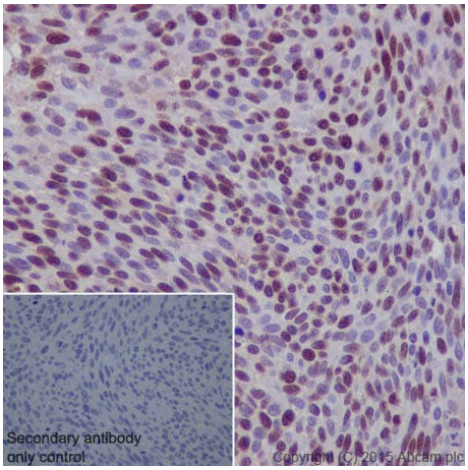
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PCNA antibody [EPR3821] (ab92552)

Immunohistochemical staining of paraffin embedded rat liver with purified ab92552 at a working dilution of 1/250. The secondary antibody used is HRP goat anti-rabbit IgG H&L (**ab97051**) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



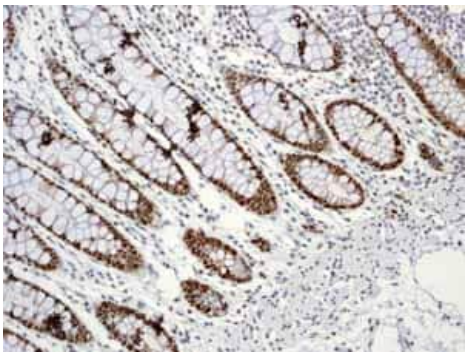
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PCNA antibody [EPR3821] (ab92552)

Immunohistochemical staining of paraffin embedded mouse testis with purified ab92552 at a working dilution of 1/250. The secondary antibody used is HRP goat anti-rabbit IgG H&L (**ab97051**) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PCNA antibody [EPR3821] (ab92552)

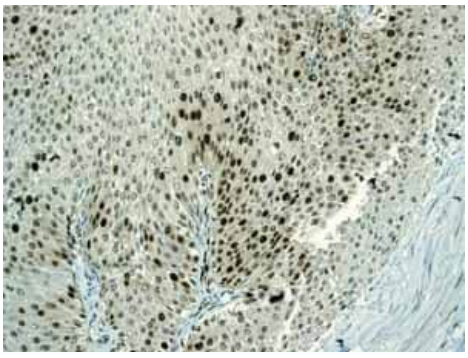
Immunohistochemical staining of paraffin embedded human cervical carcinoma with purified ab92552 at a working dilution of 1/250. The secondary antibody used is HRP goat anti-rabbit IgG H&L (**ab97051**) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PCNA antibody [EPR3821] (ab92552)

Unpurified ab92552 showing positive staining in human normal colon tissue.

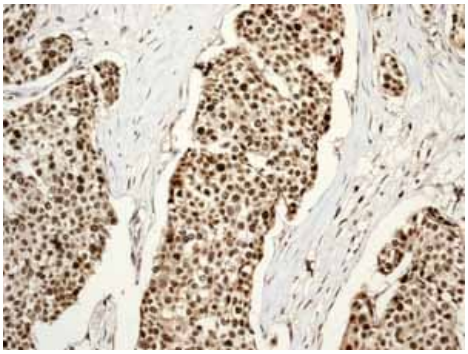
Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PCNA antibody [EPR3821] (ab92552)

Unpurified ab92552 showing positive staining in human urinary bladder carcinoma tissue.

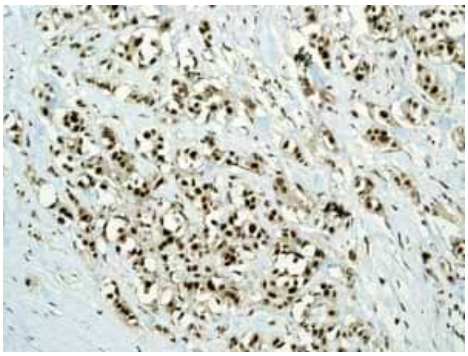
Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PCNA antibody [EPR3821] (ab92552)

Unpurified ab92552 showing positive staining in human ovarian carcinoma tissue.

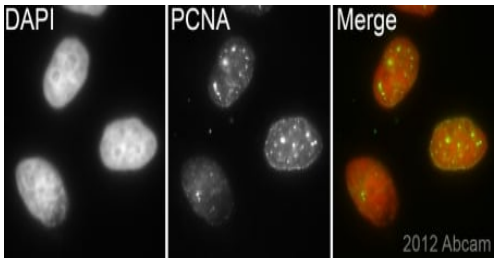
Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PCNA antibody [EPR3821] (ab92552)

Unpurified ab92552 showing positive staining in human breast carcinoma tissue.

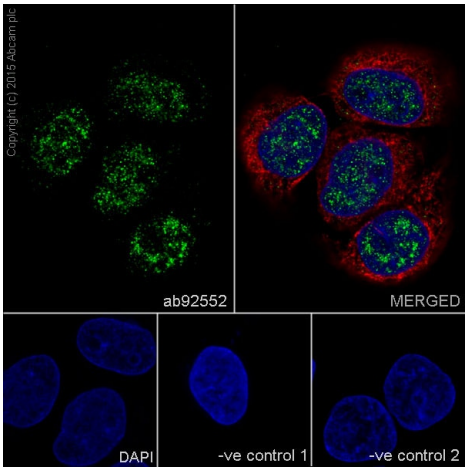
Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-PCNA antibody [EPR3821] (ab92552)

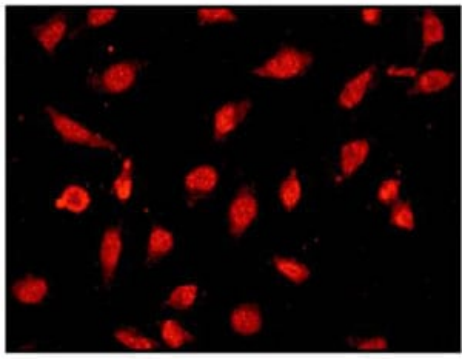
Image courtesy of an abreview submitted by Dr. Kirk McManus, Univ. of Manitoba/Cancer Care MICB, Canada

Unpurified ab92552 (1/200) staining PCNA in Hela cells (green). Cells were fixed in methanol and counterstained with DAPI in order to highlight the nucleus (red). For further experimental details please refer to abreview.



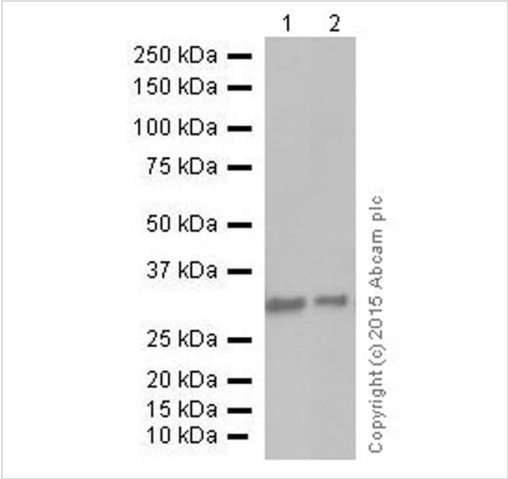
Immunocytochemistry/ Immunofluorescence - Anti-PCNA antibody [EPR3821] (ab92552)

Immunofluorescence staining of A431 cells with purified ab92552 at a working dilution of 1/100, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (**ab150077**), used at a dilution of 1/1000. **ab7291**, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with **ab150120** (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab92552 was used at a dilution of 1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody (**ab150120**) at a dilution of 1/500. For negative control 2, **ab7291** (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody (**ab150077**) at a dilution of 1/400.



Immunocytochemistry/ Immunofluorescence - Anti-PCNA antibody [EPR3821] (ab92552)

Unpurified ab92552 at 1/100 dilution staining PCNA in HeLa cells by Immunofluorescence.



Western blot - Anti-PCNA antibody [EPR3821] (ab92552)

All lanes : Anti-PCNA antibody [EPR3821] (ab92552) at 1/5000 dilution (purified)

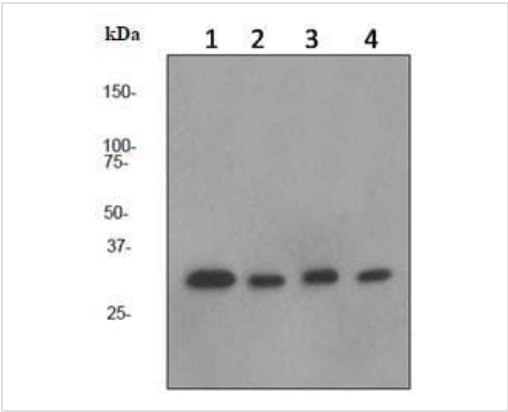
Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate
Lane 2 : HEK-293 (Human epithelial cell line from embryonic kidney) cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit IgG (H+L) at 1/20000 dilution

Predicted band size: 29 kDa
Observed band size: 29 kDa

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST



Western blot - Anti-PCNA antibody [EPR3821] (ab92552)

All lanes : Anti-PCNA antibody [EPR3821] (ab92552) at 1/50000 dilution (unpurified)

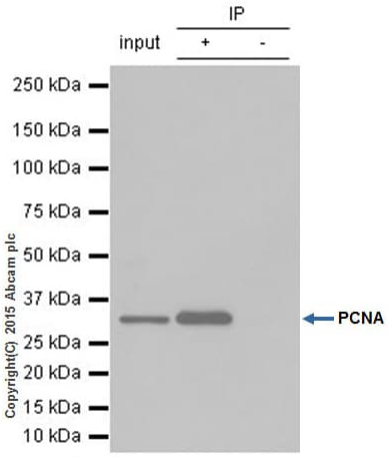
Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate
Lane 2 : HepG2 (Human liver hepatocellular carcinoma cell line) cell lysate
Lane 3 : HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) cell lysate
Lane 4 : A431 (Human epidermoid carcinoma cell line) cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

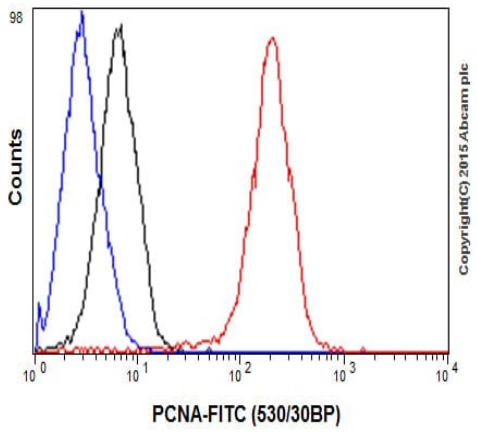
All lanes : HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 29 kDa



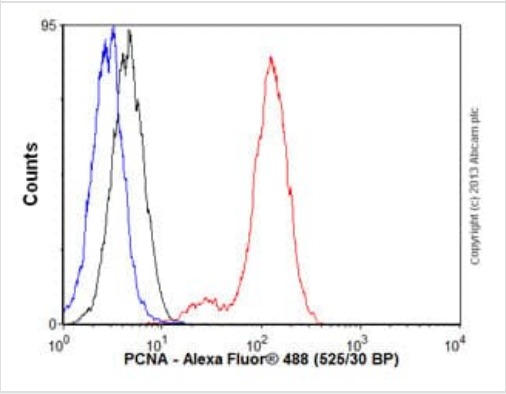
Immunoprecipitation - Anti-PCNA antibody [EPR3821] (ab92552)

ab92552 (purified) at 1/20 immunoprecipitating PCNA in 10 µg HeLa (Lanes 1 and 2, observed at 29 kDa). Lane 3 - PBS. For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10,000 dilution. Blocking buffer and concentration: 5% NFDM/TBST Dilution buffer and concentration: 5% NFDM/TBST



Flow Cytometry (Intracellular) - Anti-PCNA antibody [EPR3821] (ab92552)

Overlay histogram showing HeLa cells fixed in 4% PFA and stained with purified ab92552 at a dilution of 1 in 40 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 500. Rabbit monoclonal IgG was used as an isotype control (black line) and cells incubated in the absence of both primary and secondary antibody were used as a negative control (blue line).



Flow Cytometry (Intracellular) - Anti-PCNA antibody [EPR3821] (ab92552)

Overlay histogram showing HeLa cells stained with unpurified ab92552 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab92552, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

Anti-PCNA antibody [EPR3821] (ab92552)

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