abcam

Product datasheet

Anti-PCNA antibody [EPR3821] ab92552

ועלשעבע RabMAb

★★★★★ 12 Abreviews 354 References 画像数 17

製品の概要

製品名 Anti-PCNA antibody [EPR3821]

製品の詳細 Rabbit monoclonal [EPR3821] to PCNA

由来種 Rabbit

アプリケーション 適用あり: WB, IP, Flow Cyt (Intra), IHC-P, ICC/IF

種交差性 交差種: Mouse, Rat, Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール 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.

特記事項 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**.

製品の特性

製品の状態 Liquid

保存方法 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.

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

精製度 Protein A purified

ポリ/モノ モノクローナル

クローン名 EPR3821

アプリケーション

The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab92552の使用に適用されますアプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB	★★★★ <u>(5)</u>	1/1000 - 1/10000. Predicted molecular weight: 29 kDa.
IP		1/20 - 1/50.
Flow Cyt (Intra)		1/40. For unpurified, use 1/1000. <u>ab172730</u> - Rabbit monoclonal lgG, 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	★★★★ ☆ <u>(1)</u>	1/100 - 1/250. Use with methanol fixed samples.

ターゲット情報

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.

配列類似性

Belongs to the PCNA family.

翻訳後修飾

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.

細胞内局在

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.



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)

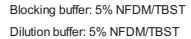
lysate

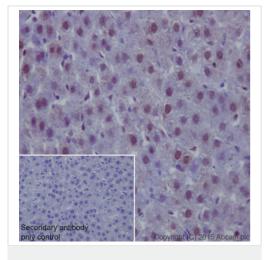
Lysates/proteins at 10 µg per lane.

Secondary

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

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

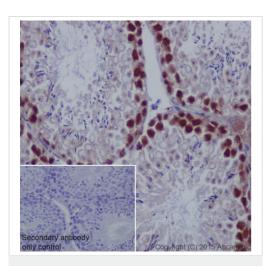




Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PCNA antibody

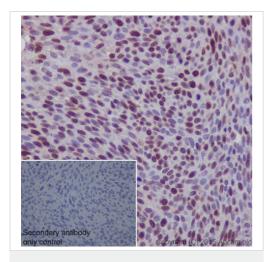
[EPR3821] (ab92552)

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 performed 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.



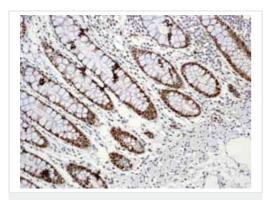
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PCNA antibody
[EPR3821] (ab92552)

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 performed 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 paraffinembedded 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 lgG H&L (ab97051) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was performed 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.

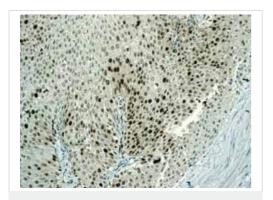


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PCNA antibody

[EPR3821] (ab92552)

Unpurified ab92552 showing positive staining in human normal colon tissue.

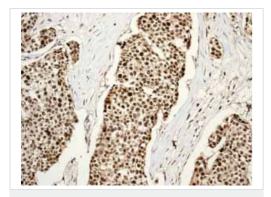
Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.



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

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

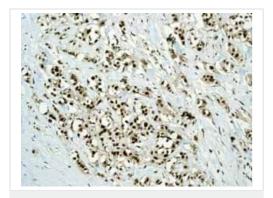
Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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.

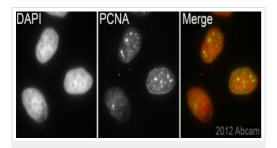


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PCNA antibody

[EPR3821] (ab92552)

Unpurified ab92552 showing positive staining in human breast carcinoma tissue.

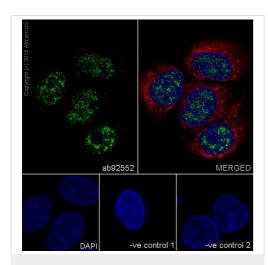
Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.



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

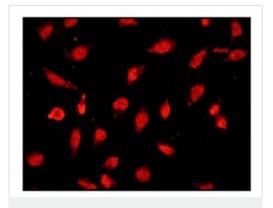
Image courtesy of an abreview submitted by Dr. Kirk McManus, Univ. of Manitoba/Cancer Care MICB, Canada

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 antitubulin 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 antitubulin) 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.

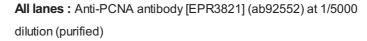


Immunocytochemistry/ Immunofluorescence - Anti-PCNA antibody [EPR3821] (ab92552)

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



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



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

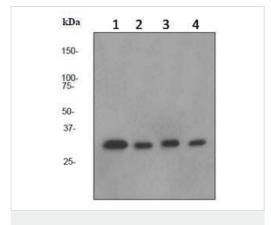
Lysates/proteins at 10 µg per lane.

Secondary

All lanes: HRP goat anti-rabbit lgG (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

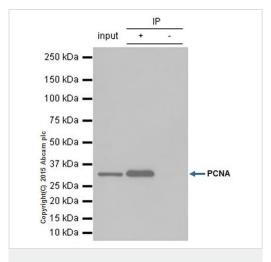
Lane 4: A431 (Human epidermoid carcinoma cell line) cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

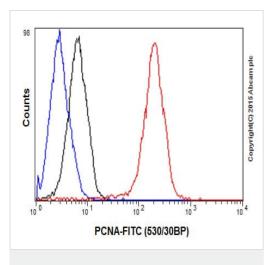
All lanes: HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 29 kDa



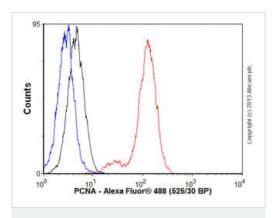
ab92552 (purified) at 1/20 immunoprecipitating PCNA in 10 μ g HeLa (Lanes 1 and 2, observed at 29 kDa). Lane 3 - PBS. For western blotting, VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10,000 dilution. Blocking buffer and concentration: 5% NFDM/TBST Dilution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

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



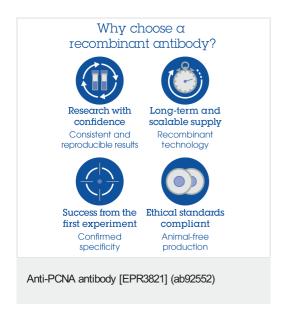
Flow Cytometry (Intracellular) - Anti-PCNA antibody [EPR3821] (ab92552)

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 Fluorr® 488 goat anti-rabbit lgG (H+L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (0.1 μ g/1x106 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.



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