Product datasheet

Anti-PCNA antibody [PC10] ab29

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Overview

Product name
Anti-PCNA antibody [PC10]

Description
Mouse monoclonal [PC10] to PCNA

Host species
Mouse

Specificity
This antibody is specific for PCNA p36 protein, expressed at high levels in proliferating cells. The epitope for the PC10 monoclonal has been mapped to amino acids 111-125 (PMID: 7680082).

Tested applications
Suitable for: IHC-P, IP, WB, ICC/IF, IHC-Fr, Flow Cyt

Species reactivity
Reacts with: Mouse, Rat, Chicken, Pigeon, Human, Pig, Drosophila melanogaster, Monkey, Zebrafish, Thrombocytoma ray, Dogfish, Catshark

Predicted to work with: Cow

Immunogen
Fusion protein corresponding to PCNA. Protein A-PCNA fusion protein obtained from pC2T construct. This construct lacked 93 nucleotides at the 3' end of PCNA.

Positive control
WB: DT40 B lymphoma cell lysate, 293 cell lysate (see review), Hela, HEK293, A431 whole cell lysates. IHC-P: mouse hippocampus (see review), Normal human tonsil, developing chick brain. IHC-Fr: rat intestine. Flow Cyt: HeLa.

General notes
This antibody clone [PC10] is manufactured by Abcam.

If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com or you can find further information here.

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 or -80°C. Avoid freeze / thaw cycle.

Storage buffer
pH: 7.40
Preservative: 0.02% Sodium azide
 Constituents: PBS, 6.97% L-Arginine

Purity
IgG fraction

Clonality
Monoclonal

Clone number
PC10

Myeloma
Sp2/0-Ag14

Isotype
IgG2a
**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.

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**Applications**

Our **Abpromise guarantee** covers the use of **ab29** in the following tested applications.

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

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/10000 - 1/30000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.</td>
</tr>
<tr>
<td>IP</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 1 µg/ml. Detects a band of approximately 30 kDa (predicted molecular weight: 29 kDa).</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 1 - 5 µg/ml. Methanol fixation recommended</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
</tr>
</tbody>
</table>
| Flow Cyt    | ⭐⭐⭐⭐⭐ | Use 1µg for 10^6 cells.  
**ab170191** - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody. |

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**Target**

**Images**
Annexin V and PCNA staining of decellularized lung scaffolds recellularized with (A) MSCs in static versus bioreactor conditions for 14 (panels A, B for annexin V, and a, b for PCNA) and 28 days (panels C, D for annexin V, and c, d for PCNA) (single cells), (B) MSC cell clusters in static conditions at 14 days (panel A for annexin V, and a for PCNA), (C) C10 cells in static (panel A for annexin V, a for PCNA) versus bioreactor (panel B for annexin V, b for PCNA) conditions for 11 days.

An inset in Fig 4Ab with higher magnification is shown to demonstrate that a majority of the cells stained positive for PCNA. Cell nuclei are labeled in blue; marker of interest is labeled in green. Magnifications are 400x. Overlap of cell nucleus and marker of interest can generate green or white color. For each condition, images are representative of the entire lung.

MSCs = Bone marrow-derived mouse mesenchymal stromal (stem) cells.

PCNA is detected using ab29 at 1/100 dilution.

(From Figure 4 of Crabbe et al)

Aldehyde dehydrogenase (ALDH1) expression correlates with clinical outcome of breast cancer patients

(A) Immunohistochemical staining shows tumors with poor clinical response (progressive or stable disease, PD/SD) to neo-adjuvant chemotherapy express high ALDH1 (>20% positive cancer cells) in pre-chemotherapy samples, and tumors with partial response (PR) express low ALDH1 (≤20% positive cancer cells). High proliferating cell nuclear antigen (PCNA) (>25% positive cancer cells) and poor apoptosis are observed in tumors with PD/SD after neo-adjuvant chemotherapy. Representative images of ALDH1 (×200), PCNA (×200) and Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP labeling (TUNEL, ×400).

PCNA is detected using ab29 at 1/100 dilution.

(From Figure 1A of Gong et al)

All lanes: Anti-PCNA antibody [PC10] (ab29) at 5 µg/ml

Lane 1: HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2: HEK293 (Human embryonic kidney cell line) Whole Cell Lysate
Lane 3: A431 (Human epithelial carcinoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes: Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Performed under reducing conditions.

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

Exposure time: 4 minutes
Immunocytochemistry/ Immunofluorescence - Anti-PCNA antibody [PC10] (ab29)

ab29 stained in Hela cells. Cells were fixed with 100% methanol (5 min) at room temperature and incubated with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% triton for 1h at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab29 at 5 µg/ml and ab6046 (Rabbit polyclonal to beta Tubulin - Loading Control) at 1/1000 dilution overnight at +4°C. The secondary antibodies were ab150117 (pseudo-colored red) and ab150080 (colored green) used at 1 µg/ml for 1 hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43 µM for 1hour at room temperature.

Immunohistochemistry (Frozen sections) - Anti-PCNA antibody [PC10] (ab29)

ab29 staining PCNA in mouse embryonic brain tissue section by Immunohistochemistry (Frozen sections). Tissue samples were fixed with paraformaldehyde and permeabilized with 0.1% PBS-Tween before blocking with 5% BSA for 1 hour at 22°C. The sample was incubated with primary antibody (1/500) in 5% BSA in 0.3% PBS-Triton-X100 for 14 hours at 22°C. An Alexa Fluor®488-conjugated Goat polyclonal to mouse IgG was used as secondary antibody at 1/500 dilution.

Flow Cytometry - Anti-PCNA antibody [PC10] (ab29)

Overlay histogram showing HeLa cells stained with ab29 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween 20 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 (ab29, 0.1µg/1x10⁶ cells) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-mouse IgG (H&L) (ab150113) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2a [ICIGG2A] (ab91361, 0.1µg/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 Laser.
ion laser (488nm) and 525/30 bandpass filter.

ab29 at 1/6000 staining mouse embryo (day 17) liver and gut tissue sections by IHC-P. The tissue was formaldehyde fixed and a heat mediated antigen retrieval step in Tris buffer was performed. The tissue was blocked before incubation with the antibody for 2 hours. A biotynlated goat polyclonal antibody was used as the secondary.

Mouse monoclonal [PC10] to PCNA - Proliferation Marker (ab29) used in immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections;1/6000 for 2h at RT) on intestine of adult Zebrafish. Antigen retrieval step: Heat mediated. Blocking step: 1% BSA for 10 mins at RT. Incubation time: Secondary Antibody: Biotin conjugated goat anti mouse Igs (1/200). NB: The crypt nuclei on this image of zebrafish intestine, are positive for the PCNA/PC10 clone conforming to accepted localisation data for PCNA in other species.

IHC image of PCNA staining in rat large intestine formalin fixed paraffin embedded tissue section, performed on a Leica Bond system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab29, 0.025µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.
Mouse monoclonal [PC10] to PCNA - Proliferation Marker (ab29) used in immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections; 1/6000 for 2h at RT) on E6 developing chick brain. Antigen retrieval step: Heat mediated. Blocking step: 1% BSA for 10 mins at RT. Incubation time: Secondary Antibody: Biotin conjugated goat anti mouse Igs (1/200). NB: This image shows developing brain/overlying skin.

Immunohistochemical staining (Formaldehyde/PFA-fixed paraffin-embedded sections) for PCNA antibody [PC10] - Proliferation Marker (ab29) on Rat Tissue sections (adult spinal cord DRG). Antigen retrieval step: Heat mediated. Blocking step: 1% BSA for 10 mins at RT. Primary Antibody used at 1/6000 for 2 minutes at RT. Secondary Antibody: Biotin labelled goat anti mouse Igs (1/200).

IHC image of PCNA staining in rat spleen formalin fixed paraffin embedded tissue section, performed on a Leica Bond system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab29, 1/30,000 dilution for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.
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