Overview

Product name  Anti-p21 antibody [EPR3993]  
Description  Rabbit monoclonal [EPR3993] to p21  
Host species  Rabbit  
Specificity  Expression levels of the target protein vary between different tissue/cell lines and in some cases induction may be required before a signal is observed. This antibody is not recommended for use in WB with tissue samples. We recommend ab109520 and ab188224 for use in IHC.  

Tested applications  Suitable for: WB, ICC/IF  
Species reactivity  Reacts with: Mouse, Rat, Human, African green monkey  
Immunogen  Synthetic peptide within Human p21 aa 100 to the C-terminus (C terminal). The exact sequence is proprietary.  
Positive control  WB: HUVEC, U87-MG, MCF-7, LnCaP, RAW264.7, PC-12, HT1080 and C6 cell lysates; Wild-type DLD-1 20 µM 2,3-DCPE for 16hrs treated cell lysate. ICC/IF: HeLa cells.  

General notes  A trial size is available to purchase for this antibody.  
Abcam recommended secondaries - Goat Anti-Rabbit HRP (ab205718) and Goat Anti-Rabbit Alexa Fluor® 488 (ab150077).  
See other anti-rabbit secondary antibodies that can be used with this antibody.  
Our RabMab® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents.  
We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.  
This product is a recombinant rabbit monoclonal antibody.  

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

Purity

Protein A purified

Clonality

Monoclonal

Clone number

EPR3993

Isotype

IgG

Applications

Our Abpromise guarantee covers the use of ab109199 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>WB</td>
<td><img src="4/5" alt="rating" /></td>
<td>1/1000. Detects a band of approximately 21 kDa (predicted molecular weight: 18 kDa). For unpurified use at 1/1000 - 1/10000. Not recommended for use with tissue samples.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td><img src="4/5" alt="rating" /></td>
<td>Use at an assay dependent concentration.</td>
</tr>
</tbody>
</table>

Target

Function

May be the important intermediate by which p53/TP53 mediates its role as an inhibitor of cellular proliferation in response to DNA damage. Binds to and inhibits cyclin-dependent kinase activity, preventing phosphorylation of critical cyclin-dependent kinase substrates and blocking cell cycle progression. Functions in the nuclear localization and assembly of cyclin D-CDK4 complex and promotes its kinase activity towards RB1. At higher stoichiometric ratios, inhibits the kinase activity of the cyclin D-CDK4 complex.

Tissue specificity

Expressed in all adult human tissues, with 5-fold lower levels observed in the brain.

Sequence similarities

Belongs to the CDI family.

Domain

The PIP-box K+4 motif mediates both the interaction with PCNA and the recruitment of the DCX(DTL) complex: while the PIP-box interacts with PCNA, the presence of the K+4 submotif recruits the DCX(DTL) complex, leading to its ubiquitination.

The C-terminal is required for nuclear localization of the cyclin D-CDK4 complex.

Post-translational modifications

Phosphorylation of Thr-145 by Akt or of Ser-146 by PKC impairs binding to PCNA. Phosphorylation at Ser-114 by GSK3-beta enhances ubiquitination by the DCX(DTL) complex. Ubiquitinated by MKRN1; leading to polyubiquitination and 26S proteasome-dependent degradation. Ubiquitinated by the DCX(DTL) complex, also named CRL4(CDT2) complex, leading to its degradation during S phase or following UV irradiation. Ubiquitination by the DCX(DTL) complex is essential to control replication licensing and is PCNA-dependent: interacts with PCNA via its PIP box, while the presence of the containing the 'K+4' motif in the PIP box, recruit the DCX(DTL) complex, leading to its degradation.

Cellular localization

Cytoplasm. Nucleus.
Lane 1: Wild-type DLD-1 cell lysate (20 µg)

Lane 2: Wild-type DLD-1 20 µM 2,3-DCPE for 16hrs treated cell lysate (20 µg)

Lane 3: p21 knockout DLD-1 cell lysate (20 µg)

Lane 4: p21 knockout 20 µM 2,3-DCPE for 16hrs DLD-1 cell lysate (20 µg)

Lane 5: HT1080 cell lysate (20 µg)

Lanes 1 - 5: Merged signal (red and green). Green - ab109199 observed at 20 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab109199 was shown to recognize p21 in WT DLD-1 cells with 2,3-DCPE treatment along with additional cross-reactive bands. When p21 knockout DLD-1 cells +/- 2,3-DCPE treatment were used, no band was observed. Wild-type and p21 knockout samples were subjected to SDS-PAGE. ab109199 and ab8245 (loading control to GAPDH) were diluted 1/1000 and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging.

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labeling p21 with ab109199 at 1/400 dilution. Cells were fixed in paraformaldehyde and permeabilized with 0.5% Triton X-100 in PBS. Staining with ab109199 at 1/400 was carried out for 1 hour at 22°C in PBS buffer. ab150081, a Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed secondary antibody was used at 1/200 dilution. DAPI was used to counterstain.
Lane 1: Anti-p21 antibody [EPR3993] (ab109199) (0.7µg/µl)
Lane 2: Anti-p21 antibody [EPR362] (ab109520) (0.8µg/µl)

All lanes: MCF-7 (Human breast adenocarcinoma epithelial cell)
whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 18 kDa

Exposure time: 3 minutes

Blocking and diluting buffer: 5% NDFM/TBST.

Lane 1: Anti-p21 antibody [EPR3993] (ab109199) (1.4µg/µl)
Lane 2: Anti-p21 antibody [EPR18021] (ab188224) (1.0µg/µl)

Lane 1: RAW264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysates
Lane 2: Neuro-2a (Mouse neuroblastoma neuroblast) whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 18 kDa
This western blot image is a comparison between ab109119 and a competitor's top cited rabbit polyclonal antibody.

**Anti-p21 antibody [EPR3993] (ab109199) at 1/1000 dilution (purified) + PC-12 cell lysate at 10 µg**

**Secondary**

Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

**Predicted band size:** 18 kDa

**Observed band size:** 21 kDa

Why is the actual band size different from the predicted?

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.

**Please note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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