abcam

Product datasheet

Anti-p21 antibody [EPR3993] - BSA and Azide free ab215971

KO VALIDATED Recombinant RabMAb

1 References 11 Images

Overview

Product name	Anti-p21 antibody [EPR3993] - BSA and Azide free
Description	Rabbit monoclonal [EPR3993] to p21 - BSA and Azide free
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 and primary cell samples. We recommended **ab109520** and **ab188224** for use in IHC.
Tested applications	**Suitable for:** WB
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** African green monkey
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	Raw 264.7, HCT116, MCF-7, PC-12 treated with 50ng/ml NFG for 48 hours whole cell lysate, wild-type HeLa Treated Fluvastatin (50 uM, 24 h) cell lysate, Wild-type DLD-1 20 µM 2,3-DCPE for 16hrs treated cell lysate
General notes	ab215971 is the carrier-free version of **ab109199**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3993
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab215971 in the following tested applications.

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

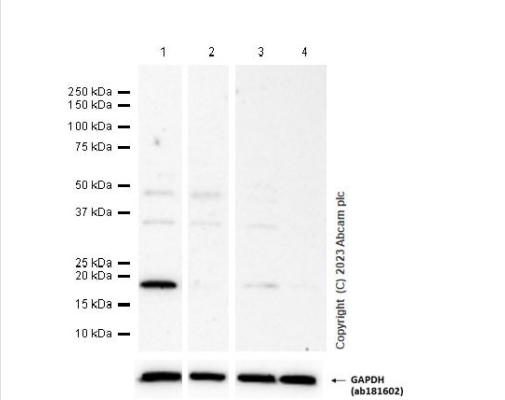
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 21 kDa (predicted molecular weight: 18 kDa).

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 recuitment 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	Phosphorylation of Thr-145 by Akt or of Ser-146 by PKC impairs binding to PCNA.

modifications	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.

Images



Western blot - Anti-p21 antibody [EPR3993] - BSA and Azide free (ab215971)

All lanes : Anti-p21 antibody [EPR3993] (**ab109199**) at 1/1000 dilution

Lane 1 : Raw 264.7(Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate
Lane 3 : C6 (Rat glial tumor glial cell) whole cell lysate
Lane 4 : PC-12(Rat adrenal gland pheochromocytoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

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

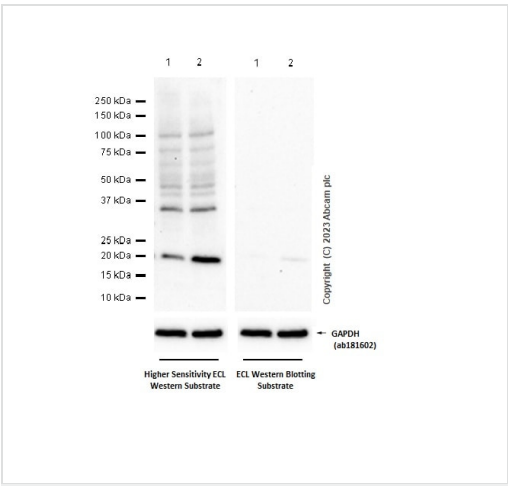
Predicted band size: 18 kDa
Observed band size: 18 kDa

Exposure time: 180 seconds

This data was developed using **ab109199**, the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

ab181602 was used as a GAPDH loading control.



Western blot - Anti-p21 antibody [EPR3993] - BSA and Azide free (ab215971)

All lanes : Anti-p21 antibody [EPR3993] (**ab109199**) at 1/1000 dilution

Lane 1 : PC-12(Rat adrenal gland pheochromocytoma) whole cell lysate
Lane 2 : PC-12(Rat adrenal gland pheochromocytoma) treated with 50ng/ml NFG for 48 hours whole cell lysate

Lysates/proteins at 20 µg per lane.

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

Developed using the ECL technique.

Predicted band size: 18 kDa
Observed band size: 18 kDa

Exposure time: 180 seconds

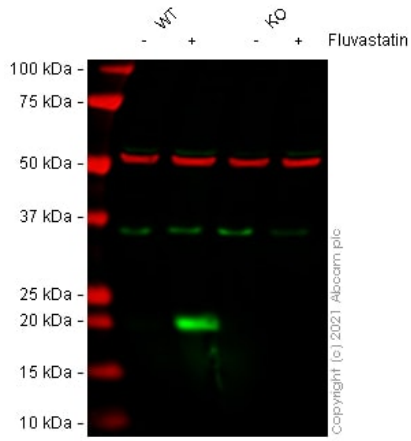
This data was developed using **ab109199**, the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

ab181602 was used as a GAPDH loading control.

We recommend using higher or super higher sensitivity ECL substrate for detecting.

Increase lysate amount can also help to get stronger signal.



Western blot - Anti-p21 antibody [EPR3993] - BSA and Azide free (ab215971)

All lanes : Anti-p21 antibody [EPR3993] (**ab109199**) at 1/1000 dilution

Lane 1 : wild-type HeLa Vehicle Control Fluvastatin (0 uM, 24 h) cell lysate
Lane 2 : wild-type HeLa Treated Fluvastatin (50 uM, 24 h) cell lysate
Lane 3 : CDKN1A knockout HeLa Vehicle Control Fluvastatin (0 uM, 24 h) cell lysate
Lane 4 : CDKN1A knockout HeLa Treated Fluvastatin (50 uM, 24 h) cell lysate

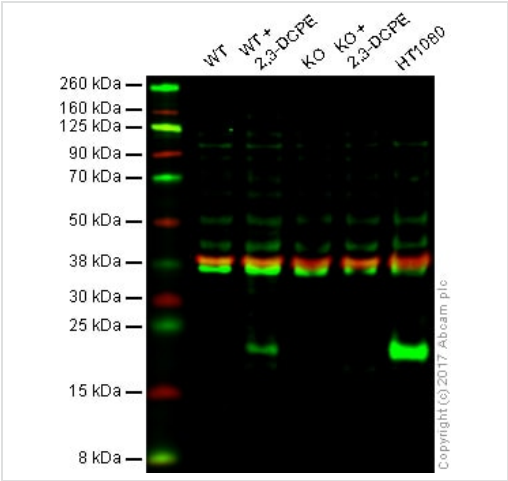
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 18 kDa
Observed band size: 21 kDa

False colour image of Western blot: Anti-p21 antibody [EPR3993] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab109199** was shown to bind specifically to p21. A band was observed at 21 kDa in wild-type y cell lysates with no signal observed at this size in CDKN2A knockout cell line **ab255349** (knockout cell lysate **ab263812**). To generate this image, wild-type and CDKN2A knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged.Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse

IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Anti-p21 antibody [EPR3993] - BSA and Azide free (ab215971)

This data was developed using **ab109199**, the same antibody clone in a different buffer formulation.

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)

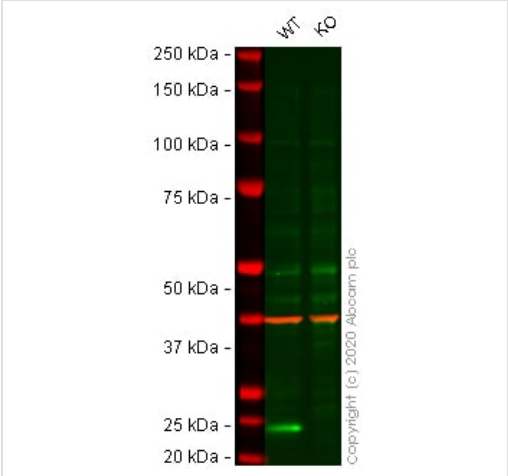
Lane 3 p21 knockout DLD-1 cell lysate (20 µg)

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.



Western blot - Anti-p21 antibody [EPR3993] - BSA and Azide free (ab215971)

All lanes : Anti-p21 antibody [EPR3993] (**ab109199**) at 1/1000 dilution

Lane 1 : Wild-type HCT116 cell lysate
Lane 2 : CDKN1A knockout HCT116 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 18 kDa
Observed band size: 20 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab109199**).

Lanes 1- 2: Merged signal (red and green). Green - **ab109199** observed at 20 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab109199 was shown to react with p21 in wild-type HCT116 cells in western blot. Loss of signal was observed when knockout cell line **ab266860** (knockout cell lysate **ab256870**) was used. Wild-type HCT116 and CDKN1A knockout HCT116 cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab109199** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. 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 in 20000 dilution for 1 hour at room temperature before imaging.

Western blot - Anti-p21 antibody [EPR3993] - BSA and Azide free (ab215971)

Lane 1 : Anti-p21 antibody [EPR3993] (**ab109199**) (0.7ug/ul)
Lane 2 : Anti-p21 antibody [EPR362] (**ab109520**) (0.7ug/ul)

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

Lysates/proteins at 15 µg per lane.

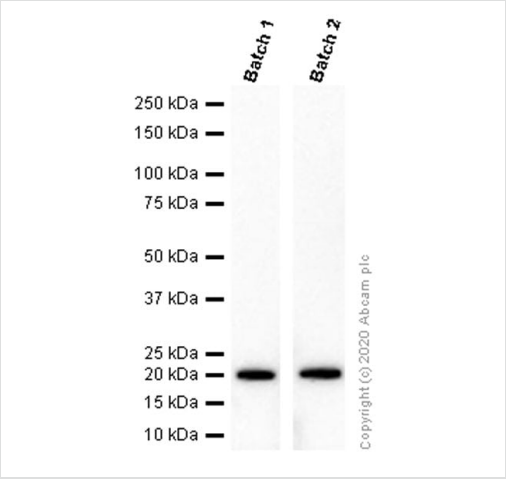
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 18 kDa

Exposure time: 3 minutes

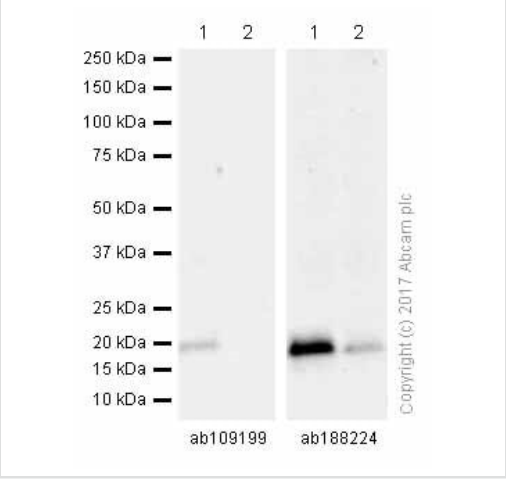
This data was developed using **ab109199**, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-p21 antibody [EPR3993] - BSA and Azide free (ab215971)

This data was developed using **ab109199**, the same antibody clone in a different buffer formulation. Different batches of **ab109199** were tested on MCF7 (Human breast adenocarcinoma epithelial cell) lysate at 0.2 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 21 kDa.



Western blot - Anti-p21 antibody [EPR3993] - BSA and Azide free (ab215971)

Lane 1 : Anti-p21 antibody [EPR3993] (**ab109199**) (1.4ug/ul)
Lane 2 : Anti-p21 antibody [EPR18021] (**ab188224**) (1.4ug/ul)

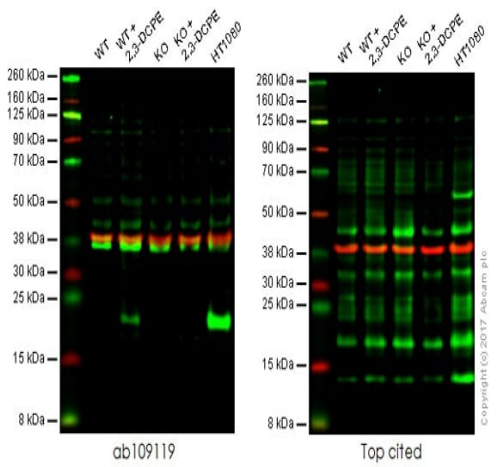
Lane 1 : RAW264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysates
Lane 2 : Neuro-2a (Mouse neuroblastoma neuroblast) whole cell lysates

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 data was developed using **ab109199**, the same antibody clone in a different buffer formulation.



Western blot - Anti-p21 antibody [EPR3993] - BSA and Azide free (ab215971)

This data was developed using **ab109199**, the same antibody clone in a different buffer formulation.

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)

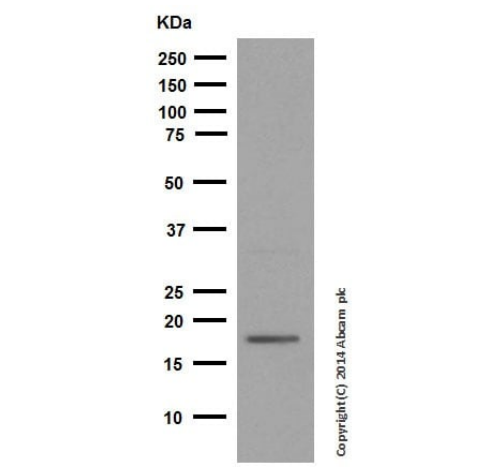
Lane 3 p21 knockout DLD-1 cell lysate (20 µg)

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.

This western blot image is a comparison between **ab109119** and a competitor's top cited rabbit polyclonal antibody.



Western blot - Anti-p21 antibody [EPR3993] - BSA and Azide free (ab215971)

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

This data was developed using **ab109199**, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.

Anti-p21 antibody [EPR3993] - BSA and Azide free (ab215971)

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