abcam

Product datasheet

Anti-p21 antibody [EPR362] - BSA and Azide free ab218311

KO VALIDATED Recombinant RabMAb

24 References 11 Images

Overview

Product name	Anti-p21 antibody [EPR362] - BSA and Azide free
Description	Rabbit monoclonal [EPR362] 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.
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, IP, WB, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: MCF7, HeLa, HEK293, HUVEC, LnCaP, U87 MG or 293T cell lysates. IHC-P: Human cervical carcinoma or papillary carcinoma of thyroid gland tissues. ICC/IF: MCF-7 cells. Flow Cyt (intra): HeLa cells. IP: HEK293 cell lysate.
General notes	ab218311 is the carrier-free version of **ab109520**. 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	EPR362
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab218311 in the following tested applications.

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

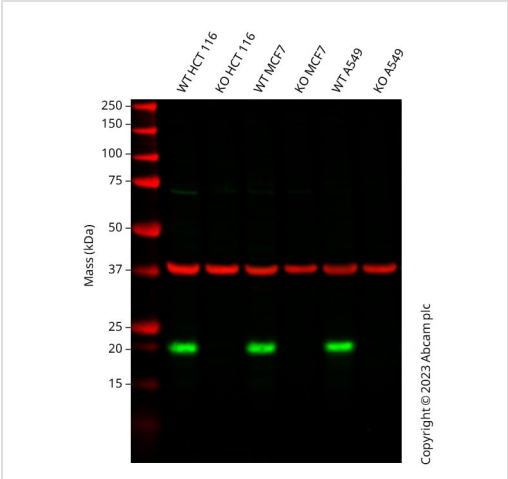
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 21 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.

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 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.

Images



Western blot - Anti-p21 antibody [EPR362] - BSA and Azide free (ab218311)

All lanes : Anti-p21 antibody [EPR362] (**ab109520**) at 1/1000 dilution

Lane 1 : Wild-type HCT 116 cell lysate
Lane 2 : CDKN1A knockout HCT 116 cell lysate
Lane 3 : Wild-type MCF7 cell lysate
Lane 4 : CDKN1A knockout MCF7 cell lysate
Lane 5 : Wild-type A549 cell lysate
Lane 6 : CDKN1A knockout A549 cell lysate

Lysates/proteins at 20 µg per lane.

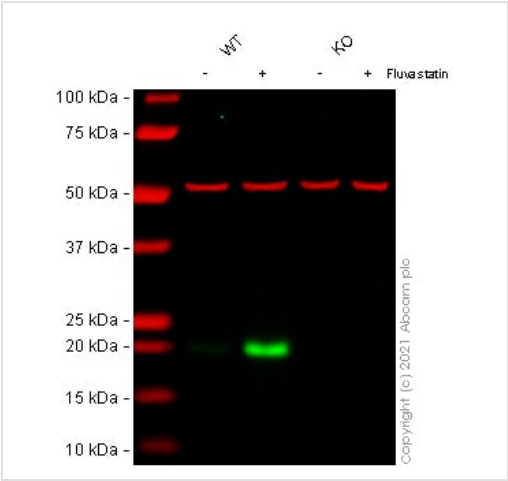
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109520**).

Western blot: Anti-CDKN1A antibody [EPR362] (**ab109520**) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab109520** was shown to bind

specifically to CDKN1A. A band was observed at 21 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in CDKN1A knockout cell line. To generate this image, wild-type and CDKN1A knockout HCT 116 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 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-p21 antibody [EPR362] - BSA and Azide free (ab218311)

All lanes : Anti-p21 antibody [EPR362] (**ab109520**) at 1/1000 dilution

Lane 1 : wild-type HeLa Vehicle Control Fluvastatin (20 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 (20 uM, 24 h) cell lysate
Lane 4 : CDKN1A knockout HeLa Treated Fluvastatin (50 uM, 24 h) cell lysate

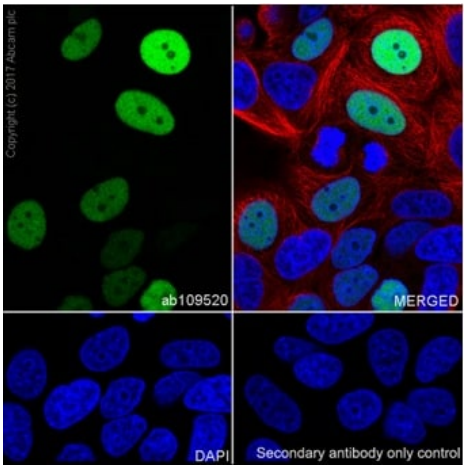
Performed under reducing conditions.

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

False colour image of Western blot: Anti-p21 antibody [EPR362] 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, **ab109520** was shown to bind specifically to p21. A band was observed at 21 kDa in wild-type HeLa cell lysates with no signal observed at this size in CDKN1A knockout cell line **ab255349** (knockout cell lysate **ab263812**). To generate this image, wild-type and CDKN1A knockout y 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.

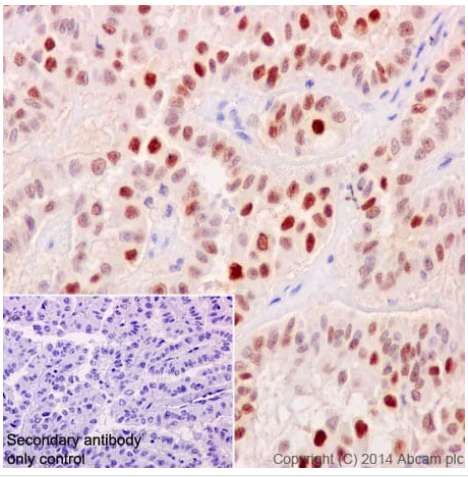
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109520**).



Immunocytochemistry/ Immunofluorescence - Anti-p21 antibody [EPR362] - BSA and Azide free (ab218311)

Cell line MCF7 (Human breast adenocarcinoma cell line), Target AbID **ab109520** anti-p21 **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary. Counterstain AbID **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)**. Fixative 4% Paraformaldehyde, Permeabilisation 0.1% tritonX-100, Nuclear counter stain DAPI. Comments Confocal image showing nuclear staining on MCF7 cell line. Target 1oAb dilution** 1:500 **2 µg/ml, Target 2ndry Ab dilution** 1:1000 **2 µg/ml, Counterstain Ab dilution** 1:200 2.5 µg/ml.

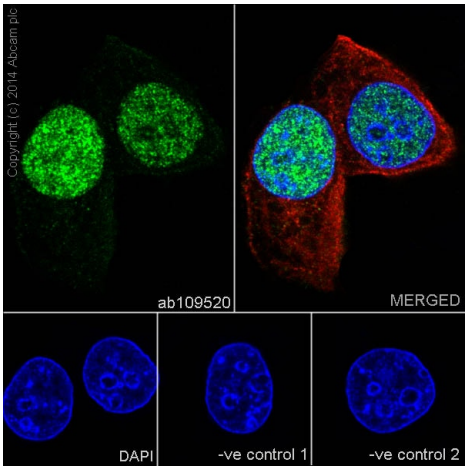
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109520**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p21 antibody [EPR362] - BSA and Azide free (ab218311)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human thyroid carcinoma tissue labelling p21 with purified **ab109520** at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109520**).



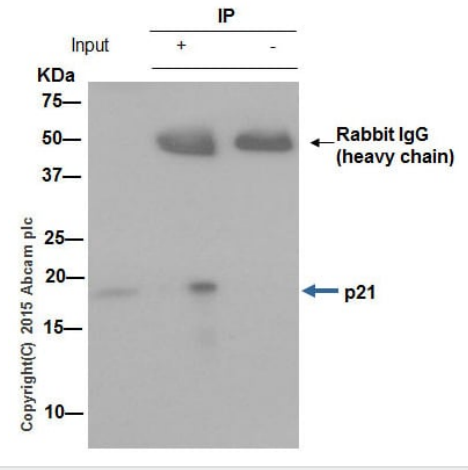
Immunocytochemistry/ Immunofluorescence - Anti-p21 antibody [EPR362] - BSA and Azide free (ab218311)

Immunocytochemistry/Immunofluorescence analysis of MCF7 cells labelling p21 with purified **ab109520** at 1/1000. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse anti-tubulin (1/1000) and **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500) were also used.

Control 1: primary antibody (1/1000) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109520**).



Immunoprecipitation - Anti-p21 antibody [EPR362] - BSA and Azide free (ab218311)

ab109520 (purified) at 1/50 immunoprecipitating p21 in HEK293 whole cell lysate.

Lane 1 (input): HEK293 whole cell lysate (10µg)

Lane 2 (+): **ab109520** + HEK293 whole cell lysate (10µg).

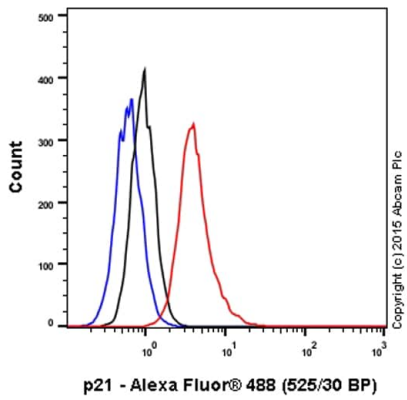
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab109520** in HEK293 whole cell lysate.

For western blotting, **ab131366** VeriBlot for IP (HRP) was used for detection at 1/1500 dilution.

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109520**).

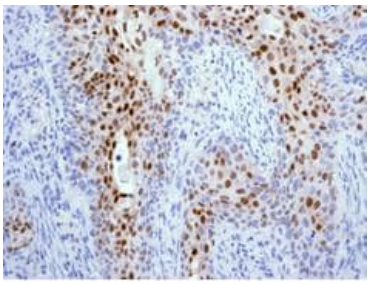


Flow Cytometry (Intracellular) - Anti-p21 antibody [EPR362] - BSA and Azide free (ab218311)

Overlay histogram showing HeLa cells stained with unpurified **ab109520** (red line). The cells were fixed with 80% methanol (5 min) 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 (unpurified **ab109520**, 1/100) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150081**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (**ab172730**, 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109520**).

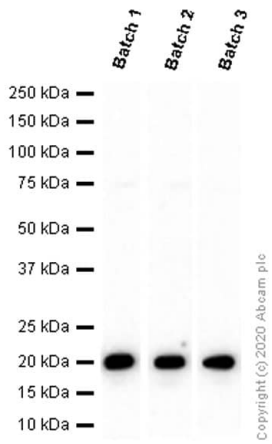


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p21 antibody [EPR362] - BSA and Azide free (ab218311)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical carcinoma tissue labelling p21 with unpurified **ab109520** at a dilution of 1/100.

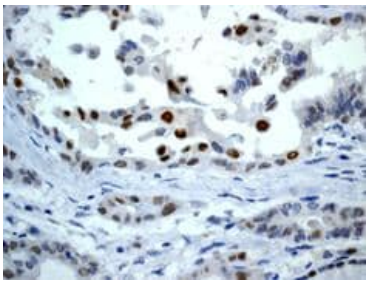
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109520**).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Western blot - Anti-p21 antibody [EPR362] - BSA and Azide free (ab218311)

This data was developed using **ab109520**, the same antibody clone in a different buffer formulation. Different batches of **ab109520** 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p21 antibody [EPR362] - BSA and Azide free (ab218311)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human papillary carcinoma of the thyroid gland tissue labelling p21 with unpurified **ab109520** at a dilution of 1/100.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109520**).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Anti-p21 antibody [EPR362] - BSA and Azide free (ab218311)

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