abcam

Product datasheet

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

KO VALIDATED | Recombinant | RabMAb

10 References 7 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:** ICC/IF, IP, WB, IHC-P, Flow Cyt
Species reactivity	**Reacts with:** Human
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: HeLa cells. IP: HEK293 cell lysate.
General notes	Ab218311 is the carrier-free version of ab109520. This format is designed for use in antibody labeling, including fluorochromes, metal isotopes, oligonucleotides, enzymes. Our carrier-free formats are supplied in a buffer free of BSA, sodium azide and glycerol for higher conjugation efficiency. 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. ab218311 is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm. *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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR362
Isotype	IgG

Applications

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.

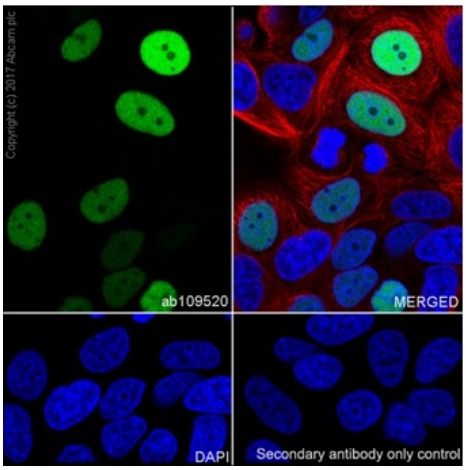
Application	Abreviews	Notes
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 21 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing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

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.

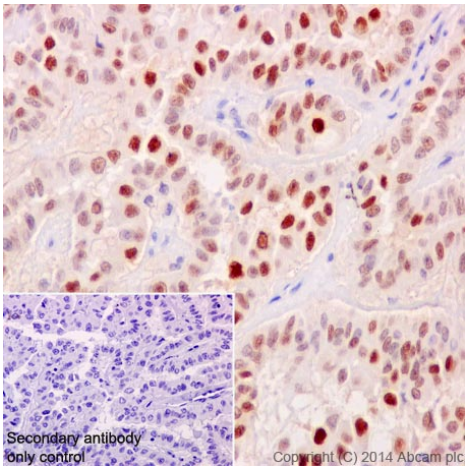
Images



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.

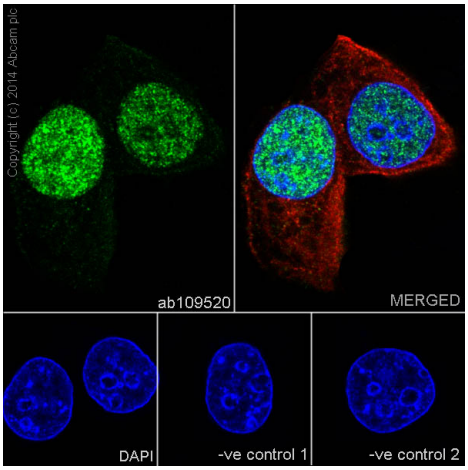
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109520).



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).



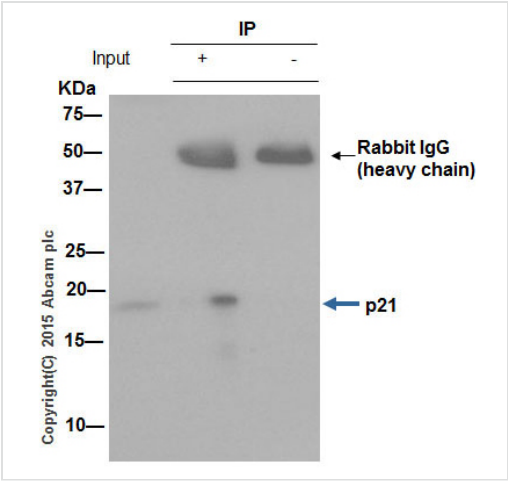
Immunocytochemistry/ Immunofluorescence - Anti-p21 antibody [EPR362] - BSA and Azide free (ab218311)

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).

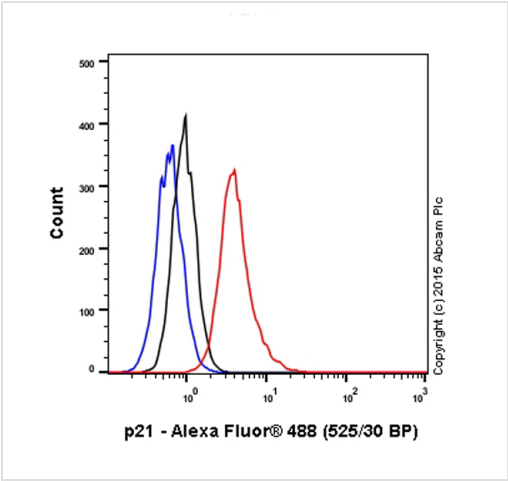
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab109520 in HEK293 whole cell lysate.

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).

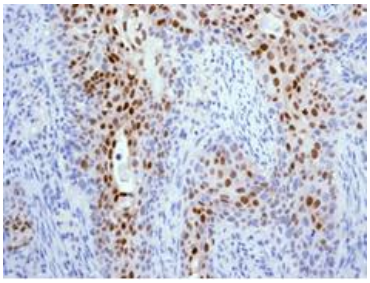


Flow Cytometry - Anti-p21 antibody [EPR362] - BSA and Azide free (ab218311)

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 22ºC. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150081) at 1/2000 dilution for 30 min at 22ºC. 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).

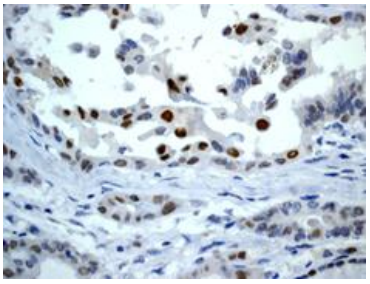


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p21 antibody [EPR362] - BSA and Azide free (ab218311)

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.



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.

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