abcam

Product datasheet

Anti-p21 antibody [EPR18021] ab188224

Recombinant RabMAb

★★★★★ 11 Abreviews 119 References 11 Images

Overview

Product name	Anti-p21 antibody [EPR18021]
Description	Rabbit monoclonal [EPR18021] 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.
Tested applications	**Suitable for:** WB, IHC-P, IP, ICC/IF, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse
Immunogen	Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: RAW 264.7 whole cell lysate; NIH/3T3, untreated and treated with 1ÂµM staurosporine for 2hrs, whole cell lysates. IHC-P: Mouse testis and lung tissues, mouse lung cancer tissue. ICC/IF: RAW 264.7 and NIH/3T3 cells. Flow Cyt (intra): RAW 264.7 cells. IP: NIN/3T3 whole cell lysate.
General notes	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: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 0.05% BSA, 40% Glycerol
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR18021
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab188224 in the following tested applications.

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

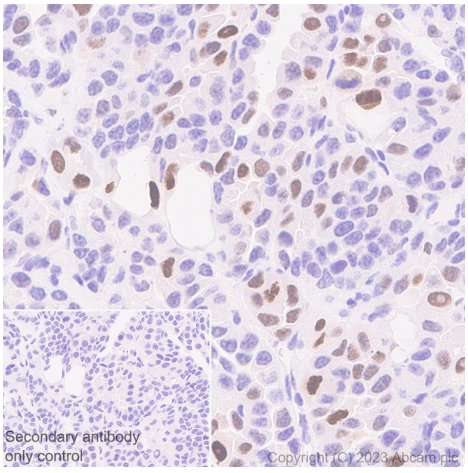
Application	Abreviews	Notes
WB	★★★★★ (1)	1/1000. Detects a band of approximately 18 kDa (predicted molecular weight: 18 kDa).
IHC-P	★★★★★ (5)	1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/30.
ICC/IF	★★★★★ (2)	1/500.
Flow Cyt (Intra)		1/50. **ab172730** - 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

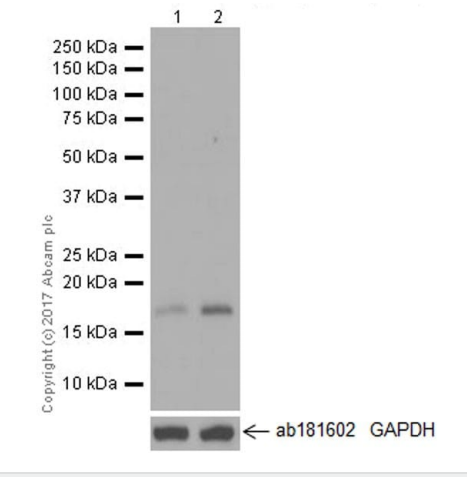


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p21 antibody [EPR18021] (ab188224)

Immunohistochemical analysis of paraffin-embedded Mouse lung cancer tissue labeling p21 with ab188224 at 1/1000 dilution (0.517 µg/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on mouse lung cancer. The section was incubated with ab188224 for 30 mins at room temperature. The section was counterstained with Hematoxylin. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Western blot - Anti-p21 antibody [EPR18021] (ab188224)

All lanes : Anti-p21 antibody [EPR18021] (ab188224) at 1/1000 dilution

Lane 1 : Untreated NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast cell line) treated with 1µM staurosporine for 2hrs, whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

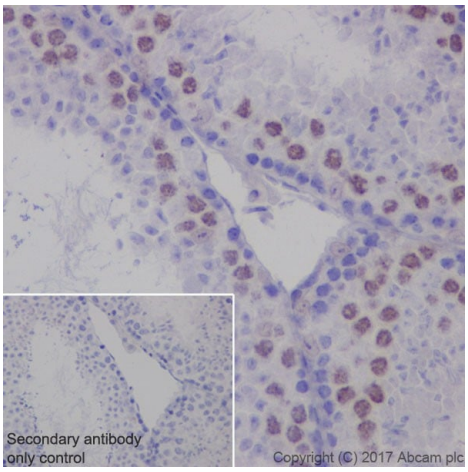
Predicted band size: 18 kDa
Observed band size: 18 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression level of p21 protein can be induced using

staurosporine (protein kinase C inhibitor) (PMID:7677742).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p21 antibody [EPR18021] (ab188224)

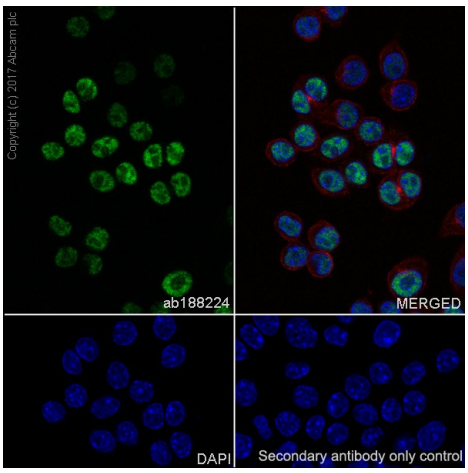
Immunohistochemical analysis of paraffin-embedded mouse testis tissue labeling p21 with ab188224 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Nuclear staining on mouse testis is observed (PMID: 9170103).

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-p21 antibody [EPR18021] (ab188224)

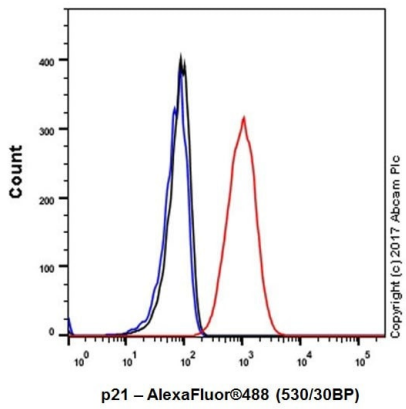
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) cells labeling p21 with ab188224 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing nuclear staining on RAW264.7 cells.

The nuclear counterstain is DAPI (blue).

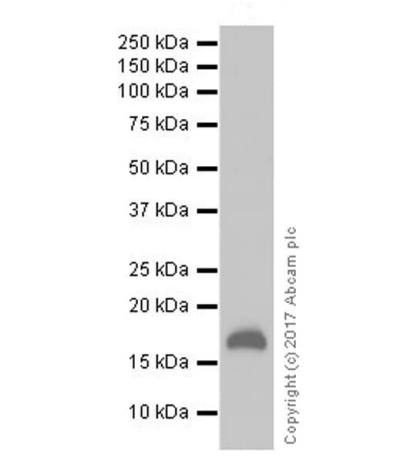
Tubulin is detected with **ab195889** (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-p21 antibody [EPR18021] (ab188224)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) cells labeling p21with ab188224 at 1/50 dilution (red) compared with a rabbit monoclonal IgG isotype control (**ab172730**; black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (Alexa Fluor® 488) (**ab150077**) at 1/2000 dilution was used as the secondary antibody.



Western blot - Anti-p21 antibody [EPR18021] (ab188224)

Anti-p21 antibody [EPR18021] (ab188224) at 1/1000 dilution + RAW264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate at 10 µg

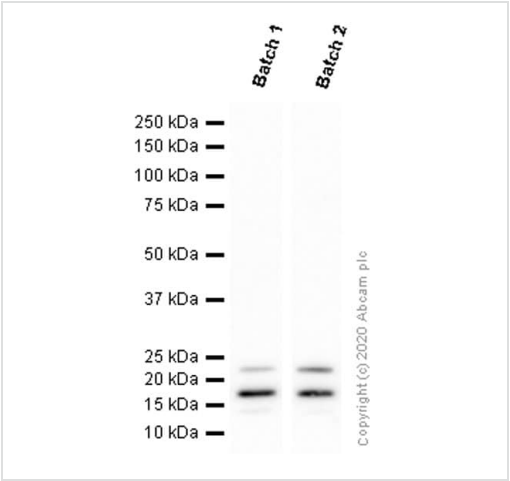
Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

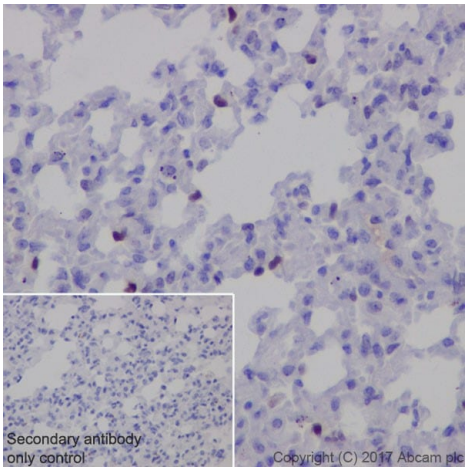
Exposure time: 8 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-p21 antibody [EPR18021] (ab188224)

Different batches of ab188224 were tested on RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) lysate at 0.1 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 18 kDa.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p21 antibody [EPR18021] (ab188224)

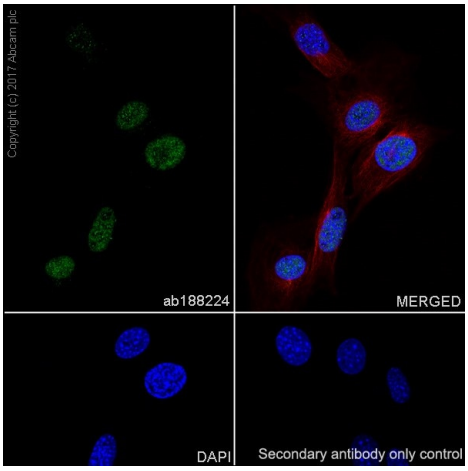
Immunohistochemical analysis of paraffin-embedded mouse lung tissue labeling p21 with ab188224 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Sporadic nuclear staining on mouse lung is observed (PMID: 25333671).

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-p21 antibody [EPR18021] (ab188224)

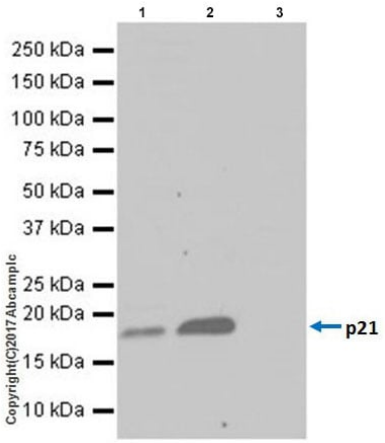
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling p21 with ab188224 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing nuclear staining on NIH/3T3 cells.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with **ab195889** (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution.



Immunoprecipitation - Anti-p21 antibody [EPR18021] (ab188224)

p21 was immunoprecipitated from 0.35 mg of NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate with ab188224 at 1/30 dilution.

Western blot was performed from the immunoprecipitate using ab188224 at 1/500 dilution.

VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1000 dilution.

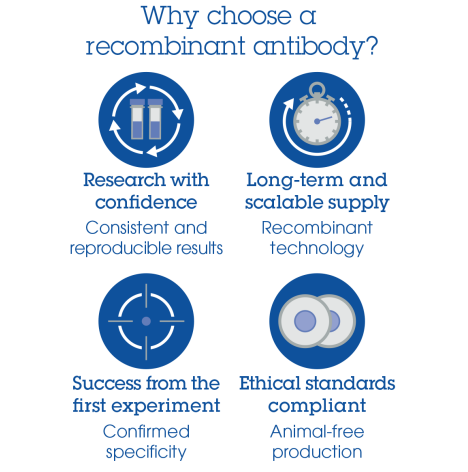
Lane 1: NIH/3T3 whole cell lysate 10 µg (Input).

Lane 2: ab188224 IP in NIH/3T3 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab188224 in NIH/3T3 whole cell lysate.

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

Exposure time: 10 seconds.



Anti-p21 antibody [EPR18021] (ab188224)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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