abcam

Product datasheet

Anti-p21 antibody [EPR18021] ab188224

重组 RabMAb

★★★★★ 11 Abreviews 119 References 11 图像

概述

产品名称	Anti-p21抗体[EPR18021]
描述	兔单克隆抗体[EPR18021] to p21
宿主	Rabbit
特异性	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.
经测试应用	**适用于:** WB, IHC-P, IP, ICC/IF, Flow Cyt (Intra)
种属反应性	**与反应:** Mouse
免疫原	Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.
阳性对照	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.
常规说明	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**.

性能

形式	Liquid
存放说明	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.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 0.05% BSA, 40% Glycerol
纯度	Protein A purified

克隆	单克隆
克隆编号	EPR18021
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab188224于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
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.

靶标

功能 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.

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

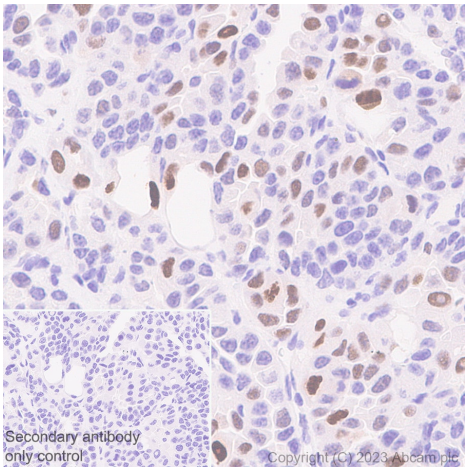
序列相似性 Belongs to the CDI family.

结构域 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.

翻译后修饰 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.

细胞定位 Cytoplasm. Nucleus.

图片

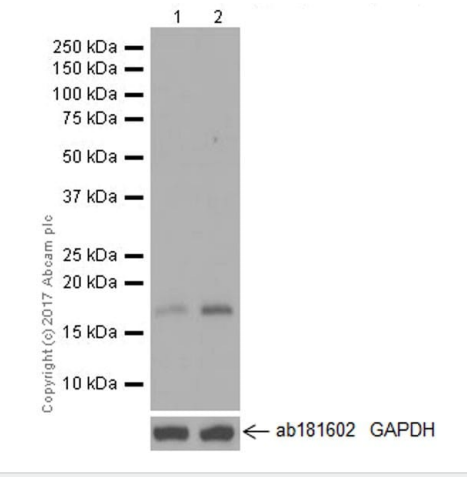


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p21 antibody [EPR18021] (ab188224)

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

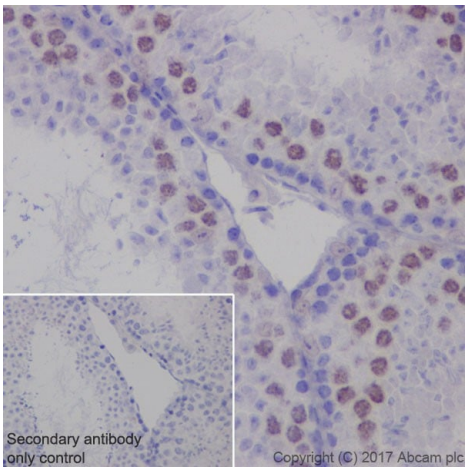
Predicted band size: 18 kDa
Observed band size: 18 kDa

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)

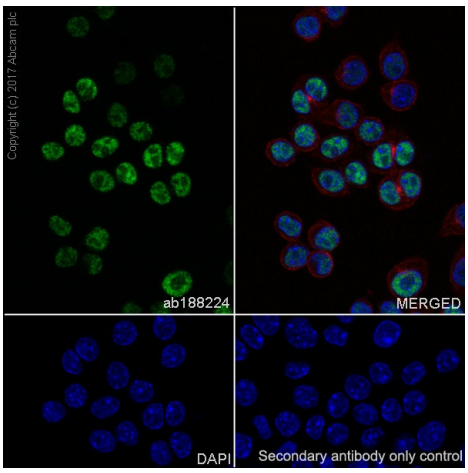
Immunohistochemical analysis of paraffin-embedded mouse testis tissue labeling p21 with ab188224 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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)

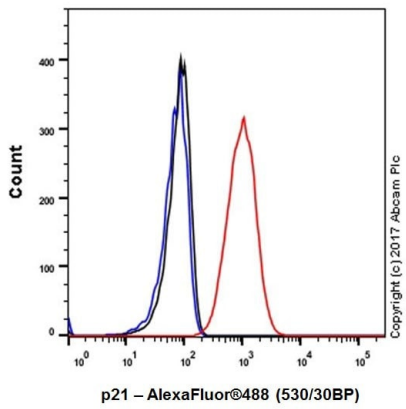
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) cells labeling p21 with ab188224 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary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

Confocal image showing nuclear staining on RAW264.7 cells.

The nuclear counterstain is DAPI (blue).

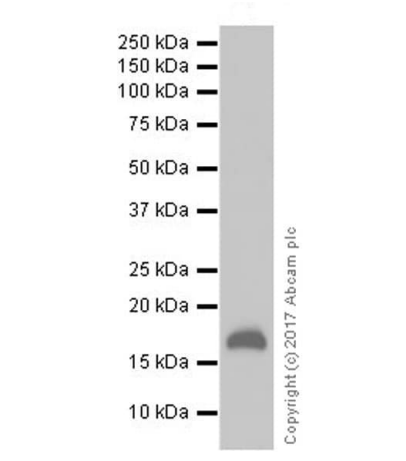
Tubulin is detected with **ab195889** (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

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

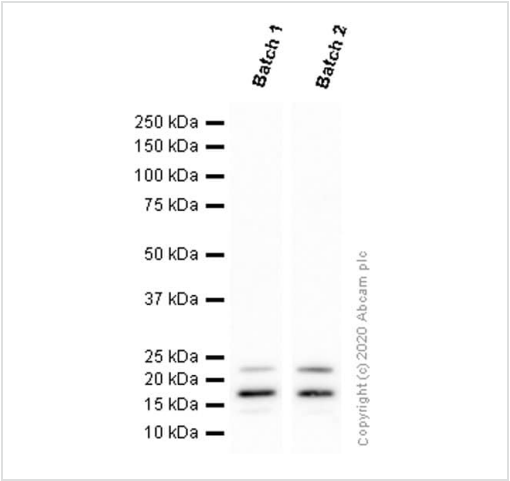
Secondary

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

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

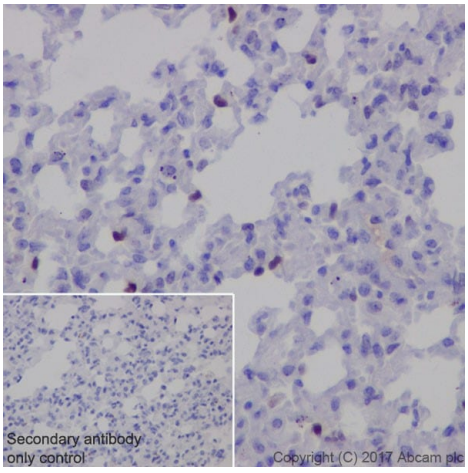
Exposure time: 8 seconds

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)

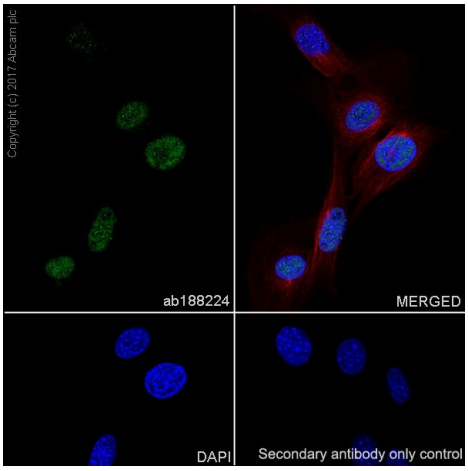
Immunohistochemical analysis of paraffin-embedded mouse lung tissue labeling p21 with ab188224 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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)

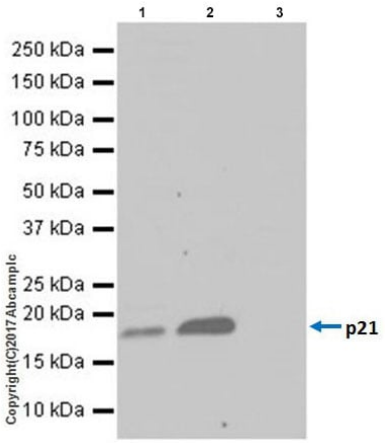
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling p21 with ab188224 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

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.

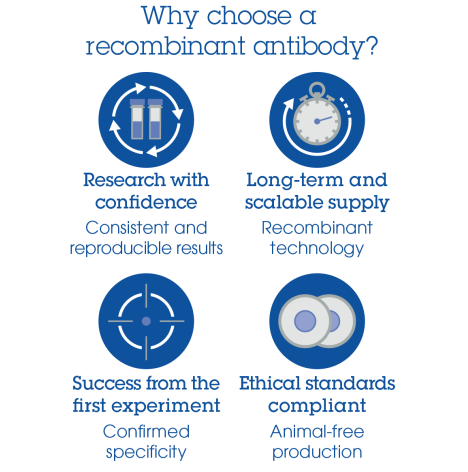
Lane 1: NIH/3T3 whole cell lysate 10 µg (Input).

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)

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