abcam

Product datasheet

Anti-p21 antibody [EPR362] ab109520

KO 評価済 リコンビナント RabMAb

14 Abreviews 331 References 画像数 16

製品の概要

製品名	Anti-p21 antibody [EPR362]
製品の詳細	Rabbit monoclonal [EPR362] to p21
由来種	Rabbit
特異性	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.
アプリケーション	**適用あり:** Flow Cyt (Intra), ICC/IF, WB, IP, IHC-P
種交差性	**交差種:** Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: MCF7, HeLa, HEK293, HUVEC, LnCaP, U87 MG or HEK-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: HEK-293 cell lysate.
特記事項	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**.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
バッファー	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 59% PBS, 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル

クローン名	EPR362
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab109520の使用に適用されます

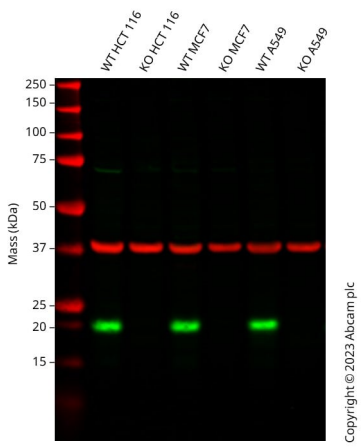
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/100.
ICC/IF	★★★★★ (3)	1/1000. **For unpurified use at 1/50 - 1/100.**
WB	★★★★☆ (5)	1/1000 - 1/10000. Predicted molecular weight: 21 kDa.
IP		1/10 - 1/100.
IHC-P	★★★★☆ (3)	1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.

ターゲット情報

機能	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.
組織特異性	Expressed in all adult human tissues, with 5-fold lower levels observed in the brain.
配列類似性	Belongs to the CDI family.
ドメイン	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.
翻訳後修飾	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.
細胞内局在	Cytoplasm. Nucleus.

画像



Western blot - Anti-p21 antibody [EPR362] (ab109520)

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

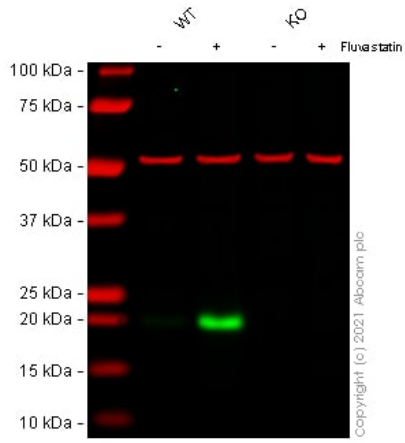
Lane 1 : Wild-type HCT 116 cell lysate
Lane 2 : CDKN1A knockout HCT 116 cell lysate
Lane 3 : Wild-type MCF7 cell lysate
Lane 4 : CDKN1A knockout MCF7 cell lysate
Lane 5 : Wild-type A549 cell lysate
Lane 6 : CDKN1A knockout A549 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

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] (ab109520)

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

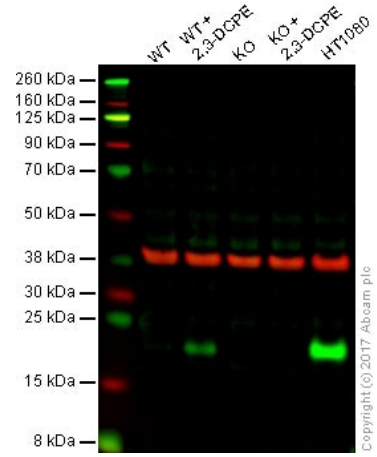
Lane 1 : wild-type HeLa Vehicle Control Fluvastatin (20 uM, 24 h) cell lysate
Lane 2 : wild-type HeLa Treated Fluvastatin (50 uM, 24 h) cell lysate
Lane 3 : CDKN1A knockout HeLa Vehicle Control Fluvastatin (20 uM, 24 h) cell lysate
Lane 4 : CDKN1A knockout HeLa Treated Fluvastatin (50 uM, 24 h) cell lysate
Lane 5 : MCF7 cell lysate
Lane 6 : SH-SY5Y 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 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 [EPR362] (ab109520)

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)

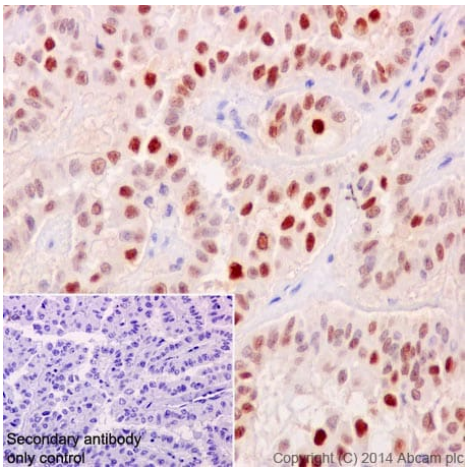
Lane 3: p21 knockout DLD-1 cell lysate (20 µg)

Lane 4: p21 knockout 20 µM 2,3-DCPE for 16hrs DLD-1 cell lysate (20 µg)

Lane 5: HT1080 cell lysate (20 µg)

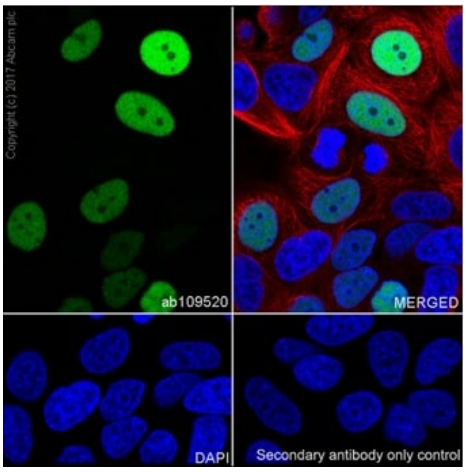
Lanes 1 - 5: Merged signal (red and green). Green - ab109520 observed at 20 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab109520 was shown to specifically recognize p21 in wild-type DLD-1 cells treated with 20 µM 2,3-DCPE. No band was observed when p21 knockout samples +/- 2,3-DCPE treatment were used. Wild-type and p21 knockout samples were subjected to SDS-PAGE. ab109520 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p21 antibody [EPR362] (ab109520)

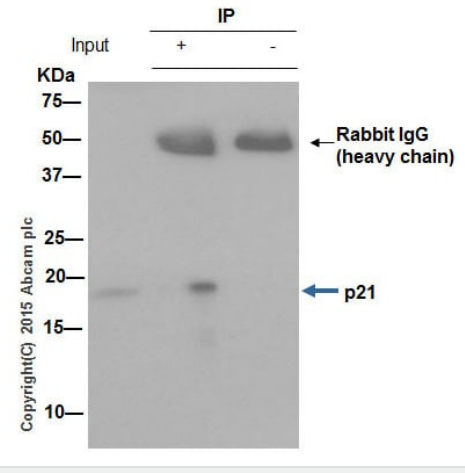
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human thyroid carcinoma tissue labelling p21 with purified ab109520 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as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



Immunocytochemistry/ Immunofluorescence - Anti-p21 antibody [EPR362] (ab109520)

Immunocytochemistry/ Immunofluorescence analysis of MCF7 (Human breast adenocarcinoma cell line) labeling p21 with ab109520 at 1/500 (2 µg/ml). **ab150077**, Alexa Fluor®488 Goat anti-Rabbit at 1/1000 (2 µg/ml) was used as secondary antibody. **ab195889**, Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200 (2.5 µg/ml) was used as counterstain AbID. Cells were fixed with 4% Paraformaldehyde and permeabilised with 0.1% tritonX-100. Nuclei were counter stained blue with DAPI.

Confocal image showing nuclear staining on MCF7 cell line.



Immunoprecipitation - Anti-p21 antibody [EPR362] (ab109520)

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

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

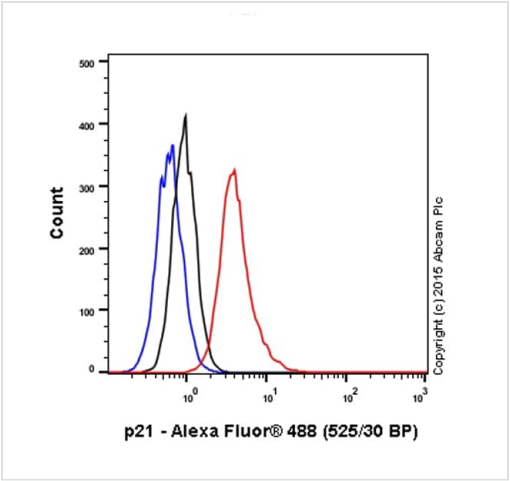
Lane 2 (+): ab109520 + HEK293 whole cell lysate (10µg).

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

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

Blocking buffer and concentration: 5% NFDM/TBST.

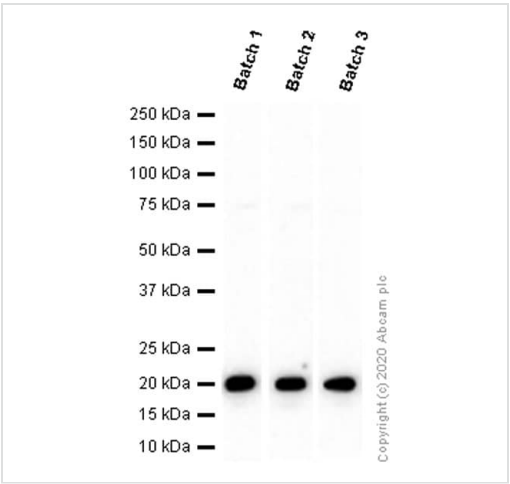
Diluting buffer and concentration: 5% NFDM /TBST.



Flow Cytometry (Intracellular) - Anti-p21 antibody [EPR362] (ab109520)

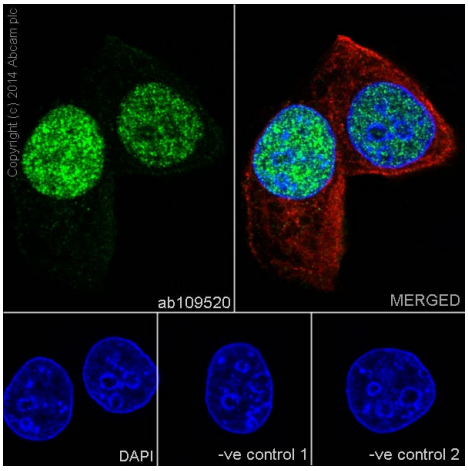
Overlay histogram showing HeLa cells stained with unpurified ab109520 (red line). The cells were fixed with 80% methanol (5 min) then permeabilized with 0.1% PBS-Tween 20 for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (unpurified ab109520, 1/100) for 30 min at 22°C. The secondary antibody used was Alexa Fluorr® 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.



Western blot - Anti-p21 antibody [EPR362] (ab109520)

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.

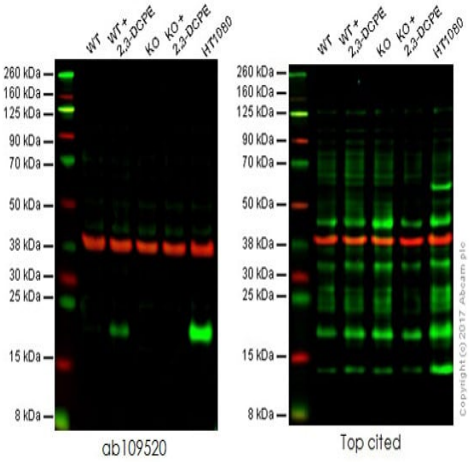


Immunocytochemistry/ Immunofluorescence - Anti-p21 antibody [EPR362] (ab109520)

Immunocytochemistry/Immunofluorescence analysis of MCF7 cells labeling 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).

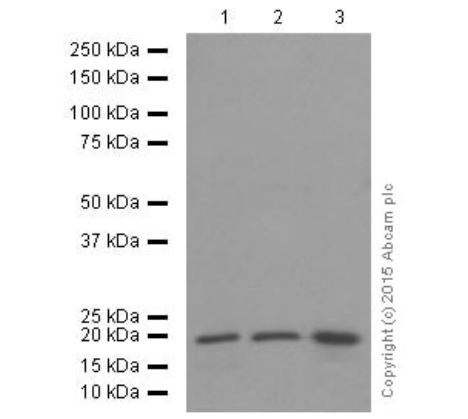


Western blot - Anti-p21 antibody [EPR362] (ab109520)

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 - ab109520 observed at 20 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

This western blot image is a comparison between ab109520 and a competitor's top cited rabbit polyclonal antibody.



Western blot - Anti-p21 antibody [EPR362] (ab109520)

All lanes : Anti-p21 antibody [EPR362] (ab109520) at 1/2000 dilution (purified)

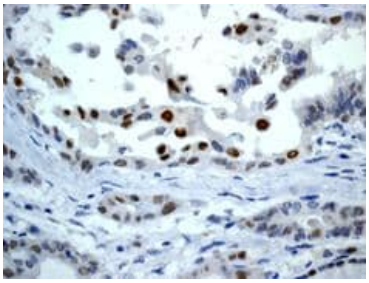
Lane 1 : MCF7 cell lysate
Lane 2 : HEK293 cell lysate
Lane 3 : U87-MG cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

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

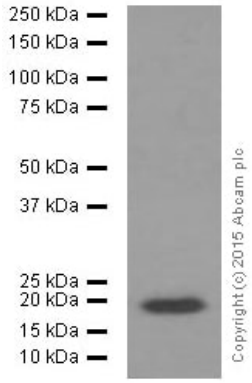
Blocking and dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p21 antibody [EPR362] (ab109520)

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.

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



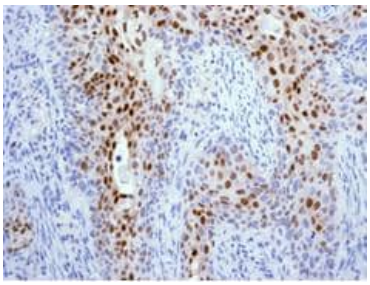
Western blot - Anti-p21 antibody [EPR362] (ab109520)

Anti-p21 antibody [EPR362] (ab109520) at 1/10000 dilution (purified) + LnCaP cell lysate at 20 µg

Secondary
Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

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

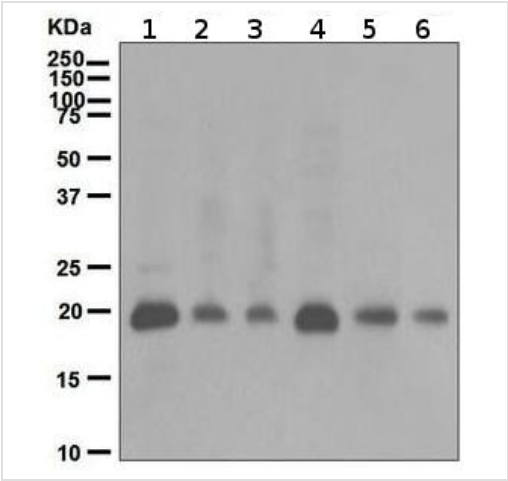
Blocking and dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p21 antibody [EPR362] (ab109520)

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

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



Western blot - Anti-p21 antibody [EPR362] (ab109520)

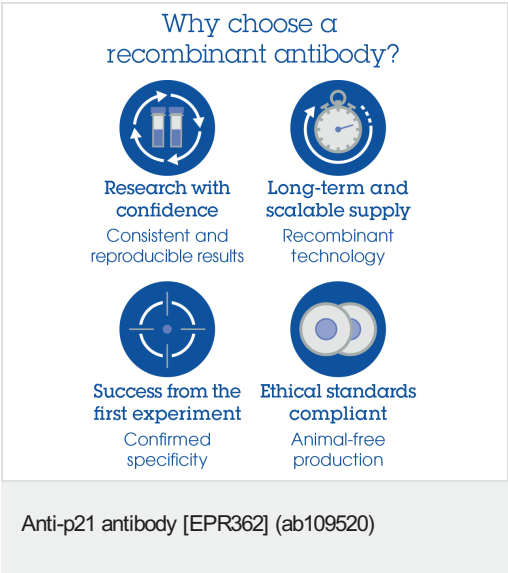
All lanes : Anti-p21 antibody [EPR362] (ab109520) at 1/1000 dilution (unpurified)

Lane 1 : MCF7 cell lysate
Lane 2 : HeLa cell lysate
Lane 3 : HUVEC cell lysate
Lane 4 : LnCap cell lysate
Lane 5 : U87 MG cell lysate
Lane 6 : 293T cell lsyate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab6721**)

Predicted band size: 21 kDa



Anti-p21 antibody [EPR362] (ab109520)

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