abcam

ab218311

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

Anti-p21 antibody [EPR362] - BSA and Azide free (ab218311) is a rabbit recombinant monoclonal antibody provided in a PBS only buffer for easy conjugation detecting p21 in **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF**. Suitable for **Human**.

- KO validated for confirmed specificity
- BSA, sodium azide, and glycerol-free for easy conjugation
- Biophysical QC for unrivalled batch-batch consistency
- Over 20 publications

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR362
Purification technique	Affinity purification Protein A
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.
Concentration	1.024 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	-
Notes	Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

IP

Tested

Species	Human
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human

Dilution info	-
Notes	-

Target data

See full target information CDKN1A

Function	Plays an important role in controlling cell cycle progression and DNA damage-induced G2 arrest (PubMed:9106657). Involved in p53/TP53 mediated inhibition of cellular proliferation in response to DNA damage. Also involved in p53-independent DNA damage-induced G2 arrest mediated by CREB3L1 in astrocytes and osteoblasts (By similarity). 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. Inhibits DNA synthesis by DNA polymerase delta by competing with POLD3 for PCNA binding (PubMed:11595739). Negatively regulates the CDK4- and CDK6-driven phosphorylation of RB1 in keratinocytes, thereby resulting in the release of E2F1 and subsequent transcription of E2F1-driven G1/S phase promoting genes (By similarity).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

What is this antibody validated in?
Anti-p21 antibody [EPR362] - BSA and Azide free (ab218311) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in Human samples.

What is the molecular weight of p21?
Anti-p21 [EPR362] - BSA and Azide free (ab218311) specifically detects a band for p21 (UniProt: P38936) at a molecular weight of 21kDa.

Trusted by the scientific community

Anti-p21 [EPR362] - BSA and Azide free (ab218311) was first used in a scientific publication in 2017 and has been cited over 20 times in peer-reviewed journals.

Specificity confirmed
The specificity of Anti-p21 antibody [EPR362] - BSA and Azide free (ab218311) has been confirmed by Western blot testing in CDKN1A Knockout HeLa cell line, ab255349.

Other related products
We have a range of other formats of antibody clone [EPR362] also available for your convenience: ab109520, Carrier free - ab218311, Alexa Fluor® 488 - ab282187

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility
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.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The p21 protein also known as CDKN1A is an important regulator of cell cycle progression. It acts as a cyclin-dependent kinase inhibitor where it binds to and inhibits the activity of cyclin-CDK2 or -CDK4 complexes. The molecular weight of p21 is approximately 21 kDa. Cellular expression of p21 is diverse; it occurs in various tissues reflecting its broad role in maintaining cell cycle control. This protein's expression level is often assessed using techniques such as p21 western blotting p21 ELISA and p21 IHC staining.
Biological function summary	P21 plays a critical role in cell cycle regulation and DNA damage response. It associates with the p53 tumor suppressor protein forming an essential part of the

	p53 signaling pathway under stress conditions. p21 arrests cells in G1 phase allowing DNA repair or leading to cellular senescence. Through these mechanisms p21 integrates signals from stress and damage emphasizing its importance as a mediator in cellular checkpoint control.
Pathways	P21 is closely involved in the p53 and the Transforming Growth Factor-beta (TGF-B) signaling pathways. This integration helps in transmitting growth-inhibitory signals. p21 interacts with proteins such as p53 and cyclins ensuring precise regulation of the cell cycle and growth arrest when necessary. Triggered by p53 activation p21 contributes to preventing undisturbed cell proliferation acting as a safeguard against tumor formation.
Associated diseases and disorders	P21's deregulation associates with cancer and age-related diseases. Its impairment is a common feature in many cancers often linked with defects in the p53 pathway. Reduced p21 expression can contribute to unchecked cell division and tumor progression. Moreover in age-related diseases p21's role in promoting cellular senescence connects it with degenerative conditions. The intricate relationship between p21 and proteins such as p53 and cyclins highlights its potential as a therapeutic target for these disorders.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

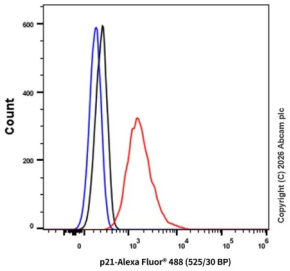
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

12 product images

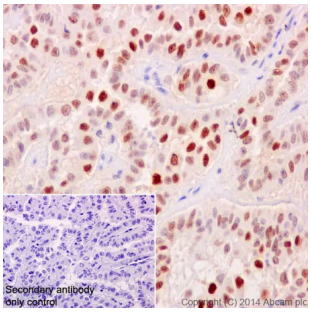


Flow Cytometry (Intracellular) - Anti-p21 antibody [EPR362] - BSA and Azide free (ab218311)

P21 Flow Cytometry (Intracellular) staining of HeLa (human cervix adenocarcinoma epithelial cell) using rabbit Anti-p21 antibody

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

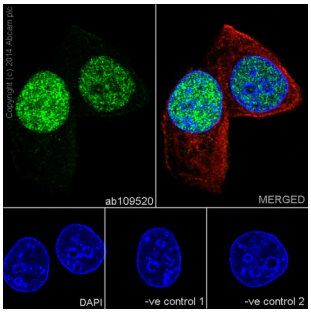
Intracellular flow cytometric analysis of 90% methanol permeabilized HeLa cells labelling p21 with ab109520 at a dilution of 1/100 (red). Cells were fixed with 4% paraformaldehyde. Goat anti-Rabbit IgG H&L (Alexa Fluor® 488, ab150081) preadsorbed (1/5000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG (ab172730). Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



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

P21 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-p21 antibody

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).



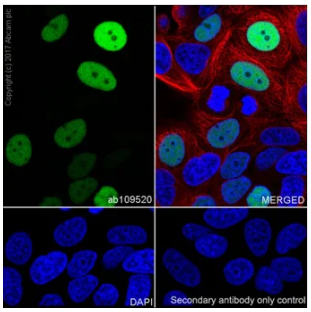
Immunocytochemistry/ Immunofluorescence - Anti-p21 antibody [EPR362] - BSA and Azide free (ab218311)

P21 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-p21 antibody

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).

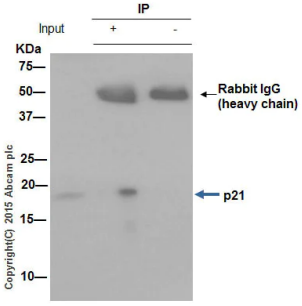
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109520).



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

P21 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-p21 antibody

Cell line MCF7 (Human breast adenocarcinoma cell line), Target AbID ab109520 anti-p21 ab150077 Alexa Fluor® 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).



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.

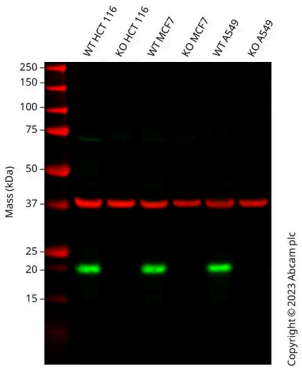
Diluting buffer and concentration: 5% NFDM /TBST.

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

All lanes:
Immunoprecipitation - Anti-p21 antibody [EPR362] (ab109520)

Predicted band size: 18 kDa

Observed band size: 21 kDa



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

P21 Western blot staining using rabbit Anti-p21 antibody

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

Lane 1:
Wild-type HCT 116 cell lysate at 20 µg

Lane 2:
CDKN1A knockout HCT 116 cell lysate at 20 µg

Lane 3:
Wild-type MCF7 cell lysate at 20 µg

Lane 4:
CDKN1A knockout MCF7 cell lysate at 20 µg

Lane 5:
Wild-type A549 cell lysate at 20 µg

Lane 6:
CDKN1A knockout A549 cell lysate at 20 µg

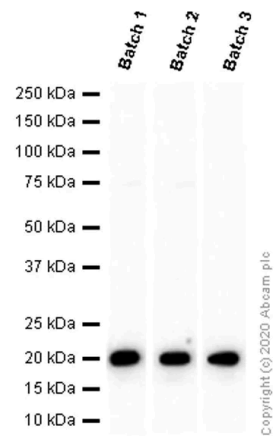
Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 21 kDa

Observed band size: 21 kDa

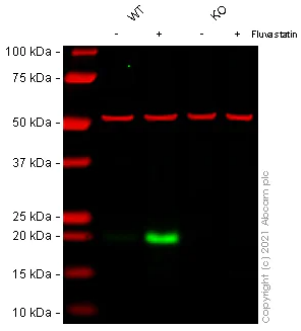


Western blot - Anti-p21 antibody [EPR362] - BSA and Azide free (ab218311)

P21 Western blot staining using rabbit Anti-p21 antibody

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.
All lanes:
Western blot - Anti-p21 antibody [EPR362] (ab109520)

Predicted band size: 18 kDa



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

P21 Western blot staining using rabbit Anti-p21 antibody

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

Lane 1:
wild-type HeLa Vehicle Control Fluvastatin (20 uM, 24 h) cell lysate at 20 µg

Lane 2:
wild-type HeLa Treated Fluvastatin (50 uM, 24 h) cell lysate at 20 µg

Lane 3:
CDKN1A knockout HeLa Vehicle Control Fluvastatin (20 uM, 24 h) cell lysate at 20 µg

Lanes 3 - 4:
Western blot - Human CDKN1A knockout HeLa cell line (ab255349)

Lane 4:
CDKN1A knockout HeLa Treated Fluvastatin (50 uM, 24 h) cell lysate at 20 µg

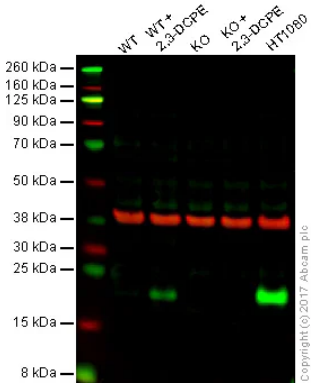
Lane 5:
MCF7 cell lysate at 20 µg

Lane 6:
SH-SY5Y cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 18 kDa

Observed band size: 21 kDa



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

P21 Western blot staining using rabbit Anti-p21 antibody

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

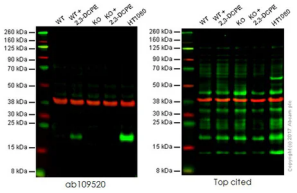
Lane 5: HT1080 cell lysate (20 µg)

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.
All lanes:
Western blot - Anti-p21 antibody [EPR362] (ab109520)

Predicted band size: 18 kDa

Observed band size: 20 kDa



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

P21 Western blot staining using rabbit Anti-p21 antibody

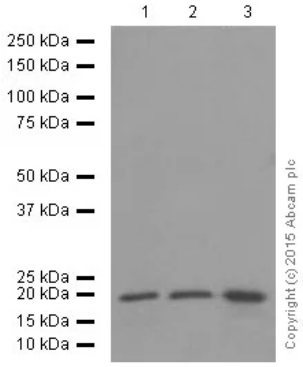
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109520).
Lane 1: Wild-type DLD-1 cell lysate (20 µg)
Lane 2: Wild-type DLD-1 20 µM 2,3-DCPE for 16hrs treated cell lysate (20 µg)
Lane 3: p21 knockout DLD-1 cell 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.

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

Predicted band size: 18 kDa



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

P21 Western blot staining using rabbit Anti-p21 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109520).
Blocking and dilution buffer: 5% NFDM/TBST.

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

Lane 1:
MCF7 cell lysate at 20 µg

Lane 2:
HEK293 cell lysate at 20 µg

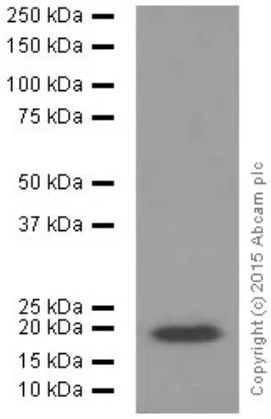
Lane 3:
U87-MG cell lysate at 20 µg

Secondary

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

Predicted band size: 18 kDa, 24 kDa, 57 kDa

Observed band size: 21 kDa



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

P21 Western blot staining of LnCaP cell lysate using rabbit Anti-p21 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109520).
Blocking and dilution buffer: 5% NFDM/TBST.

All lanes:
Western blot - Anti-p21 antibody [EPR362] (ab109520) at 1/10000 dilution

All lanes:
LnCaP cell lysate at 20 µg

Secondary

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

Predicted band size: 18 kDa

Observed band size: 21 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.