abcam

ab108349

Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal

Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349) is a rabbit monoclonal antibody detecting CDKN2A/p16INK4a in **Western Blot, Flow Cytometry (Intra), IP, IHC-P**. Suitable for **Human**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 190 publications

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR1473
Purification technique	Affinity purification Protein A
Specificity	Expression levels of the CDKN2A/p16INK4a protein may vary with sample type. It is barely expressed in normal tissue, and mostly expressed in some tumour tissues, such as cervical cancer, breast cancer and so on. Moreover, only expressed in some cell lines. Please see images for recommended positive controls.
Concentration	1.311 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/30
Notes	**For unpurified use at 1/10 - 1/100.**

WB

Tested

Species	Human
Dilution info	1/2000
Notes	**For unpurified use at 1/1000 - 1/10000.**

ICC/IF

Not recommended

Species	Human
Dilution info	-
Notes	**For unpurified use at 1/100 - 1/250.**

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/270 - 1/500
Notes	For unpurified use at 1/100 - 1/500. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

IHC-P

Tested

Species	Human
Dilution info	1/100
Notes	**For unpurified use at 1/250 - 1/500.** Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target data

See full target information CDKN2A

Function	Acts as a negative regulator of the proliferation of normal cells by interacting strongly with CDK4 and CDK6. This inhibits their ability to interact with cyclins D and to phosphorylate the retinoblastoma protein.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt (Intra), IHC-P, IP and WB.

Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349) was first used in a scientific publication in 2013 and has been cited over 192 times in peer reviewed journals. It's performance in Western Blot and IHC in human samples is trusted by the scientific community.

Abcam's high quality manufacturing and validation processes ensure Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.

The specificity of Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349) has been confirmed by Western Blot testing in CDKN2A/p16INK4a knockout HEK-293T cells.

Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349) has 6 independent reviews from customers.

Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349) specifically detects CDKN2A/p16INK4a (UniProt ID: P42771; Molecular weight: 17kDa) and is sold in a convenient trial size to enable initial testing (20 µL) and larger sizes for subsequent scaling up experiments (100 µL and 1 mL).

Conjugation-ready, carrier free format available for antibody clone EPR1473 - ab186932.

Antibody clone EPR1473 is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor® 647, PE (ab192054, ab209579).

CDKN2A, encoding the tumor suppressor protein p16INK4a, plays a crucial role in regulating the cell cycle by inhibiting cyclin-dependent kinases, thereby preventing uncontrolled cell proliferation. Mutations or deletions in CDKN2A are frequently associated with various cancers, including melanoma, pancreatic cancer and head and neck squamous cell carcinoma.

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.

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.

13 product images

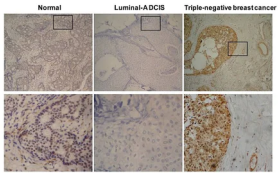
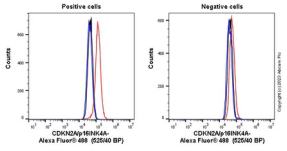


Image from Shan M et al., PLoS One. 2013;8(10):e76408. Fig 1.; doi: 10.1371/journal.pone.0076408. eCollection 2013. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349)

Formalin-fixed, paraffin-embedded human normal breast, luminal-A DCIS (ductal carcinoma *in situ*) and triple negative breast cancer tissues stained for CDKN2A/p16INK4a using ab108349 in immunohistochemical analysis.



Flow Cytometry (Intracellular) - Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349)

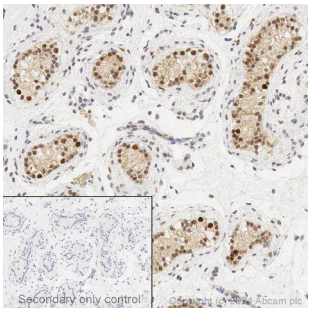
CDKN2A/p16INK4a Flow Cytometry (Intracellular) staining using rabbit Anti-CDKN2A/p16INK4a antibody

Flow cytometry overlay histogram showing left HeLa positive cells and right negative MCF7 stained with ab108349 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody ab108349 (1/500) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



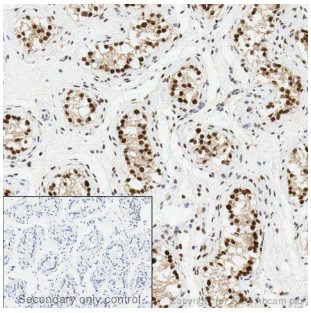
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349)

CDKN2A/p16INK4a Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human testis using rabbit Anti-CDKN2A/p16INK4a antibody

Immunohistochemical analysis of formalin fixed paraffin embedded human testis labelling CDKN2A/p16INK4a with ab108349 at a concentration of 1µg/ml. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with a Bond™ Polymer Refine Detection kit. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution 2) for 20mins.

ab108349 anti-CDKN2A/p16INK4a antibody [EPR1473] was incubated for 15mins at room temperature. Sections were counterstained with Hematoxylin. Image inset shows absence of staining in secondary antibody only control.

Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual)



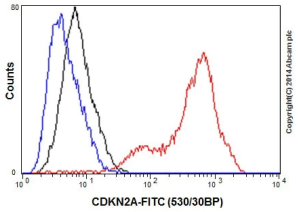
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349)

CDKN2A/p16INK4a Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human testis using rabbit Anti-CDKN2A/p16INK4a antibody

Immunohistochemical analysis of formalin fixed paraffin embedded human testis labelling CDKN2A/p16INK4a with ab108349 at a concentration of 0.5µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with a OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was performed with DISCOVERY cell conditioning solution (CC1) 100°C, pH8.5 for 32mins.

ab108349 anti-CDKN2A/p16INK4a antibody [EPR1473] was incubated for 16mins at 37°C. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

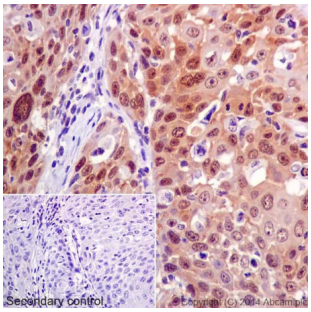
Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual)



Flow Cytometry (Intracellular) - Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349)

CDKN2A/p16INK4a Flow Cytometry (Intracellular) staining using rabbit Anti-CDKN2A/p16INK4a antibody

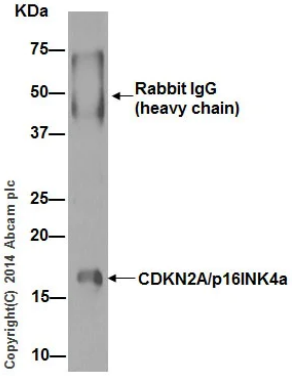
Intracellular Flow Cytometry analysis of HEK-293 (Human epithelial cell line from embryonic kidney) cells labeling CDKN2A/p16INK4a with purified ab108349 at 1/270 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabeled control, cells without incubation with primary and secondary antibodies.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349)

CDKN2A/p16INK4a Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CDKN2A/p16INK4a antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervix carcinoma tissue labeling CDKN2A/p16INK4a with purified ab108349 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, an 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.



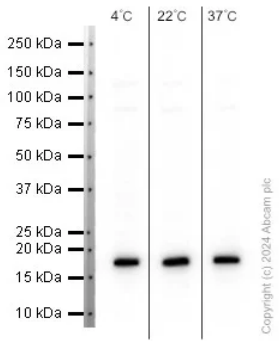
Immunoprecipitation - Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349)

ab108349 (purified) at 1/30 immunoprecipitating CDKN2A/p16INK4a in HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate. For western blotting, a peroxidase-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/1000).
Blocking/Dilution buffer: 5% NFDM/TBST.

All lanes:
Immunoprecipitation - Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349)

Predicted band size: 17 kDa

Observed band size: 17 kDa



Western blot - Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349)

CDKN2A/p16INK4a Western blot staining of HeLa whole cell lysate using rabbit Anti-CDKN2A/p16INK4a antibody

This blot was produced using 4-20% SDS-PAGE containing 15 µg of HeLa whole cell lysate per lane at 150V for 1hr before being transferred onto a 0.45 µm PVDF membrane at 75V for 1hr. The membrane was then blocked for 1hr using 5% NFDM/TBST, then incubated with ab108349 (1/1000) at room temperature for 1hr. After being washed three times in TBST, the membrane was

incubated with Peroxidase conjugated goat anti-rabbit IgG (H+L) (ab97051) at 1/20,000 dilution for 1hr at room temperature. The membrane was washed three times again. Then the signal was developed using the ECL technique.

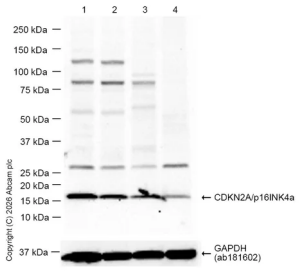
ab108349 was stored at a range of temperatures (+4°C, +22°C, +37°C) for 1 week before being tested in WB. The image shows the band intensity remains relatively constant across all storage temperatures, demonstrating that antibody activity is not affected under these conditions. This data was generated as part of a structured antibody stability study supporting Abcam's transition from cold⊠chain to ambient shipping.
All lanes:
Western blot - Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349) at 1/1000 dilution

All lanes:
HeLa whole cell lysate at 15 µg with NDFM/TBST

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Exposure time: 7s



Western blot - Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349)

CDKN2A/p16INK4a Western blot staining using rabbit Anti-CDKN2A/p16INK4a antibody

In Western blot, anti-GAPDH antibody (ab181602) loading control staining at 1/1000000 dilution.

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

All lanes:
Western blot - Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349) at 1/1000 dilution

Lane 1:
HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
293T (human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Lane 3:
Saos-2 (human osteosarcoma epithelial) whole cell lysate at 20 µg

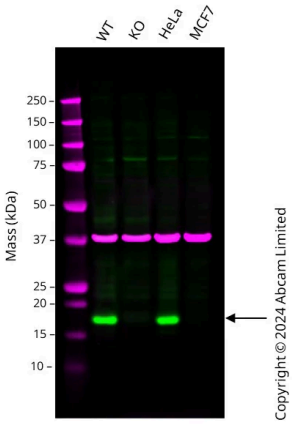
Lane 4:
HUVEC (human umbilical vein endothelial cell) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 16 kDa, 36 kDa

Exposure time: 120s



Western blot - Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349)

CDKN2A/p16INK4a Western blot staining using rabbit Anti-CDKN2A/p16INK4a antibody

Western blot: Anti-CDKN2A antibody [EPR1473] (ab108349) staining at 1/4000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab108349 was shown to bind specifically to CDKN2A. A band was observed at 17 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in CDKN2A knockout cell line. To generate this image, wild-type and CDKN2A knockout HEK-293T cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % 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-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349) at 1/4000 dilution

Lane 1:
Wild-type HEK-293T cell lysate at 20 µg

Lane 2:
CDKN2A knockout HEK-293T cell lysate at 20 µg

Lane 3:
HeLa cell lysate at 20 µg

Lane 4:
MCF7 cell lysate at 20 µg

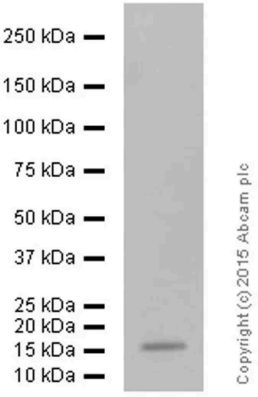
Secondary

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: 17 kDa

Observed band size: 17 kDa



Western blot - Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349)

CDKN2A/p16INK4a Western blot staining of HEK293 (human embryonic kidney) whole cell lysate using rabbit Anti-CDKN2A/p16INK4a antibody

Blocking buffer and concentration: 5% NFDM/TBST
Diluting buffer and concentration: 5% NFDM/TBST

All lanes:
Western blot - Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349)

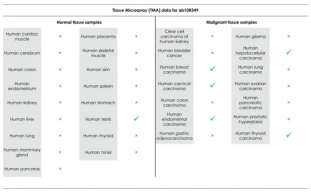
All lanes:
HEK293 (human embryonic kidney) whole cell lysate at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051)

Predicted band size: 17 kDa

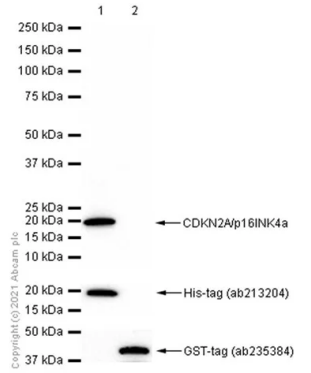
Exposure time: 3min



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349)

CDKN2A/p16INK4a Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CDKN2A/p16INK4a antibody

Tissue Microarrays stained for CDKN2A/p16INK4a using ab108349 in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes. The sections were incubated with ab108349 for 30 mins at room temperature followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Western blot - Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349)

CDKN2A/p16INK4a Western blot staining using rabbit Anti-CDKN2A/p16INK4a antibody

5% NFDM/TBST was used as a blocking and diluting buffer.
All lanes:
Western blot - Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349) at 1/1000 dilution

Lane 1:
Human CDKN2A full-length recombinant protein with His-tag at 0.01 µg

Lane 2:
Human CDKN2B full-length recombinant protein with GST-tag at 0.01 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 17 kDa

Observed band size: 17 kDa

Exposure time: 10s

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