abcam

ab186932

Anti-CDKN2A/p16INK4a antibody [EPR1473] - BSA and Azide free

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	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	1.048 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Not recommended

Species	Human
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	Please check the parent abID, ab108349, for a recommended dilution.

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

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

Notes

ab186932 is the carrier-free version of ab108349.

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.

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

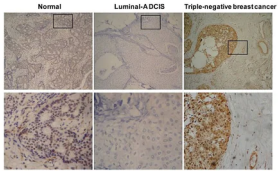
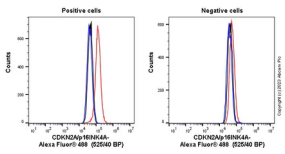


Image from Shan M et al., PLoS One. 2013;8(10):e76408. Fig 1.; doi: 10.1371/journal.pone.0076408. eCollection 2013. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CDKN2A/p16INK4a antibody [EPR1473] - BSA and Azide free (ab186932)

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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab108349).



Flow Cytometry (Intracellular) - Anti-CDKN2A/p16INK4a antibody [EPR1473] - BSA and Azide free (ab186932)

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

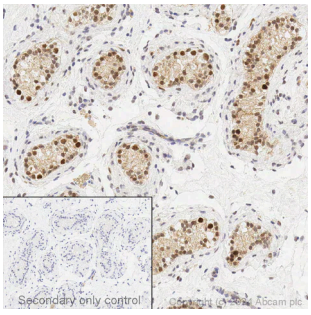
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab108349).

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.



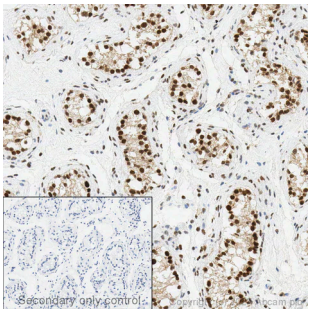
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CDKN2A/p16INK4a antibody [EPR1473] - BSA and Azide free (ab186932)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab108349).
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] - BSA and Azide free (ab186932)

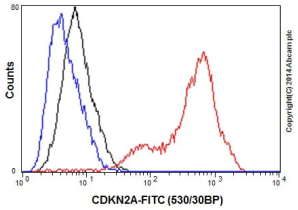
CDKN2A/p16INK4a Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human testis using rabbit Anti-CDKN2A/p16INK4a antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab108349).
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.

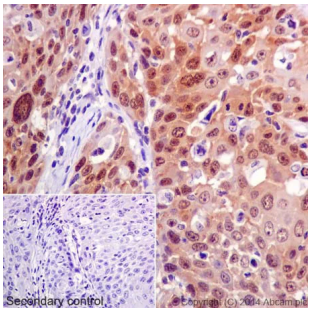
Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual)



Flow Cytometry (Intracellular) - Anti-CDKN2A/p16INK4a antibody [EPR1473] - BSA and Azide free (ab186932)

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

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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab108349).

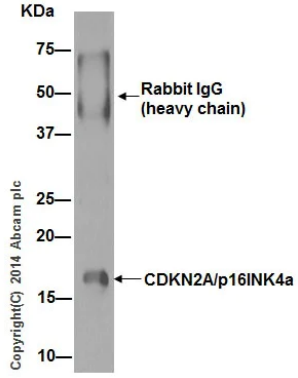


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CDKN2A/p16INK4a antibody [EPR1473] - BSA and Azide free (ab186932)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab108349).



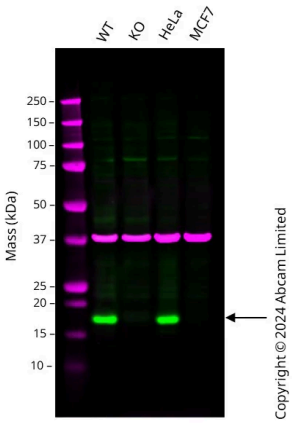
Immunoprecipitation - Anti-CDKN2A/p16INK4a antibody [EPR1473] - BSA and Azide free (ab186932)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab108349).
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] - BSA and Azide free (ab186932)

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

This data was developed using the same antibody clone in a different buffer formulation (ab108349).

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

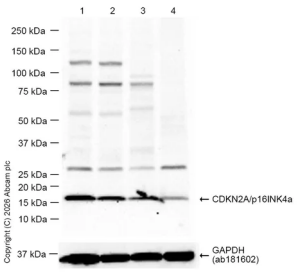
Secondary

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] - BSA and Azide free (ab186932)

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

This data was developed using ab108349, the same antibody clone in a different buffer formulation.

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

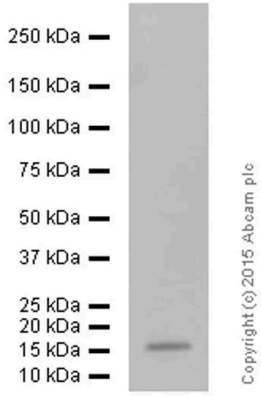
Lane 4:
HUVEC (human umbilical vein endothelial cell) whole cell lysate at 20 µg

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] - BSA and Azide free (ab186932)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab108349).
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)

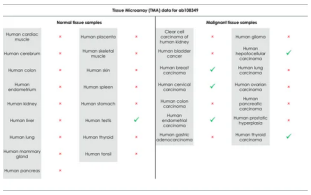
All lanes:
HEK293 (human embryonic kidney) whole cell lysate at 10 µg

Secondary

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

Predicted band size: 17 kDa

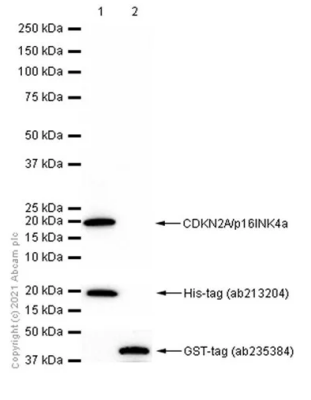
Exposure time: 3min



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CDKN2A/p16INK4a antibody [EPR1473] - BSA and Azide free (ab186932)

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] - BSA and Azide free (ab186932)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab108349).
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'.