abcam

Product datasheet

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

Recombinant RabMAb

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Overview

Product name	Anti-CDKN2A/p16INK4a antibody [EPR1473] - BSA and Azide free
Description	Rabbit monoclonal [EPR1473] to CDKN2A/p16INK4a - BSA and Azide free
Host species	Rabbit
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.
Tested applications	**Suitable for:** WB, Flow Cyt (Intra), IHC-P, IP **Unsuitable for:** ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	HeLa, 293T, and Saos-2 cell lysates, Human cervical carcinoma tissue, HeLa cells Flow cyto(intra): HeLa cells.
General notes	ab186932 is the carrier-free version of **ab108349**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. 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. 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. 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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR1473
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab186932 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 17 kDa. Please check the parent abID, **ab108349**, for a recommended dilution.
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P	★★★★☆ **(1)**	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
IP		Use at an assay dependent concentration.

Application notes Is unsuitable for ICC/IF.

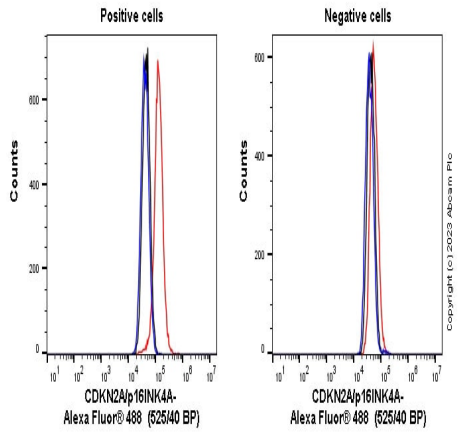
Target

Cellular localization Cytoplasmic and Nuclear

Form

There are 4 isoforms produced by alternative splicing. Isoform 1 also known as: p16INK4a; Isoform 3 also known as: p12; Isoform 4 also known as: p14ARF; p19ARF; ARF.

Images



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

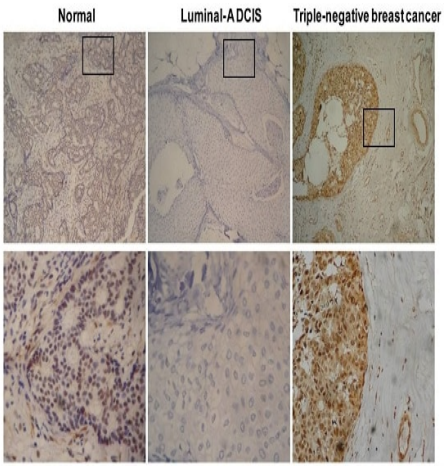
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108349**).

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 permeabilised 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**) (1x 10^6 in 100µl at 0.04µg/ml (1/52500)) 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.



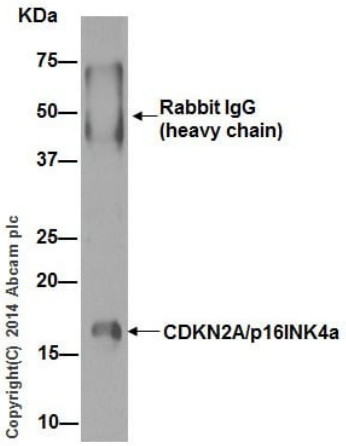
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CDKN2A/p16INK4a antibody [EPR1473] - BSA and Azide free (ab186932)

Image from Shan M et al.,; PLoS One. 2013;8(10):e76408. Fig 1.; doi: 10.1371/journal.pone.0076408. eCollection 2013.

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**).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

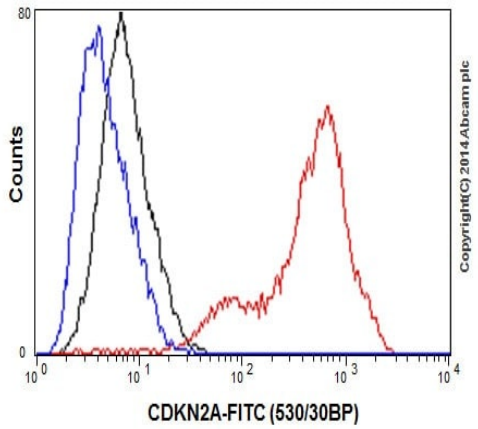


Immunoprecipitation - Anti-CDKN2A/p16INK4a antibody [EPR1473] - BSA and Azide free (ab186932)

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.

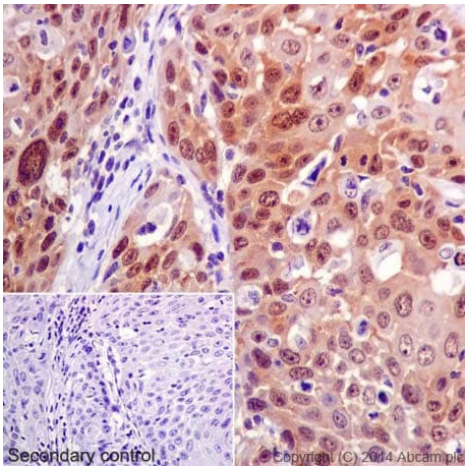
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108349**).



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

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.

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

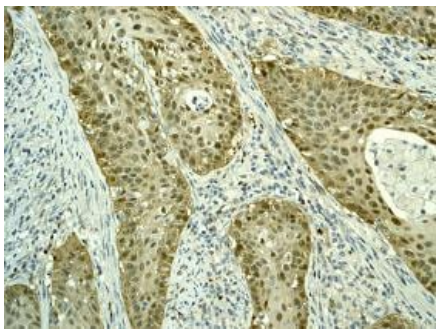


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CDKN2A/p16INK4a antibody [EPR1473] - BSA and Azide free (ab186932)

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**).

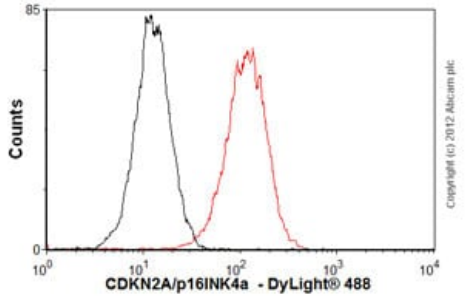


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CDKN2A/p16INK4a antibody [EPR1473] - BSA and Azide free (ab186932)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical carcinoma tissue labeling CDKN2A/p16INK4a with unpurified **ab108349** at a dilution of 1/250.

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

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

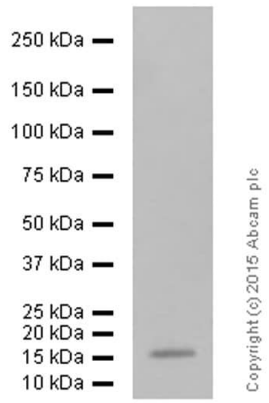


Flow Cytometry (Intracellular) - Anti-CDKN2A/p16INK4a antibody [EPR1473] - BSA and Azide free (ab186932)

Overlay histogram showing HEK-293 (Human epithelial cell line from embryonic kidney) cells stained with unpurified **ab108349** (red line). The cells were fixed with 4% paraformaldehyde (10 minutes) and then permeabilized with 0.1% PBS-Tween for 20 minutes. 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 (**ab108349**, 1/100) for 30 minutes at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (**ab96899**) at 1/500 dilution for 30 minutes at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HEK-293 cells fixed with 80% methanol (5 minutes)/permeabilized with 0.1% PBS-Tween for 20 minutes used

under the same conditions.

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



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

Anti-CDKN2A/p16INK4a antibody [EPR1473] - BSA and Azide free (ab186932) + HEK293 (human embryonic kidney) whole cell lysate at 10 µg

Secondary

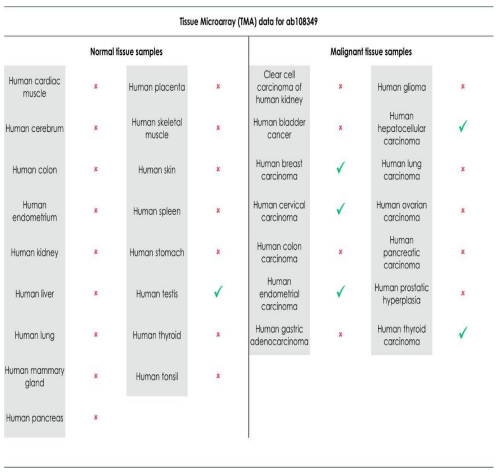
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**)

Predicted band size: 17 kDa

Exposure time: 3 minutes

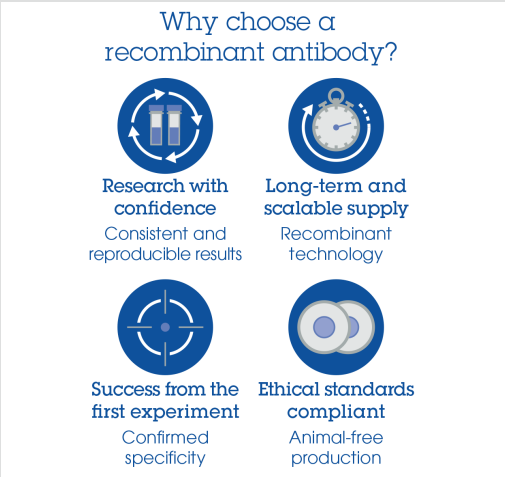
Blocking buffer and concentration: 5% NFDM/TBST

Diluting buffer and concentration: 5% NFDM/TBST



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

Tissue Microarrays stained for " Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal" 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.



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

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