abcam

Product datasheet

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

Recombinant RabMAb

7 Abreviews 162 References 13 Images

Overview

Product name	Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal
Description	Rabbit monoclonal [EPR1473] to CDKN2A/p16INK4a - C-terminal
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, IP, IHC-P, Flow Cyt (Intra) **Unsuitable for:** ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, HEK-293, HEK-293T, and Saos-2 cell lysates, His-tagged human CDKN2A recombinant protein lysates, human CDKN2A full-length recombinant protein with His-tag, human CDKN2B full-length recombinant protein with GST-tag. IHC-P: Human cervical carcinoma tissue. ICC/IF: HeLa cells. Flow Cyt (intra): HEK-293 and HeLa cells. IP: HeLa cell lysate.
General notes	Abcam recommended secondaries - Goat Anti-Rabbit HRP (**ab205718**) and Goat Anti-Rabbit Alexa Fluor® 488 (**ab150077**). Or search our wide range of secondary antibodies for use with your experiment. 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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, PBS, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR1473
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab108349 in the following tested applications.

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

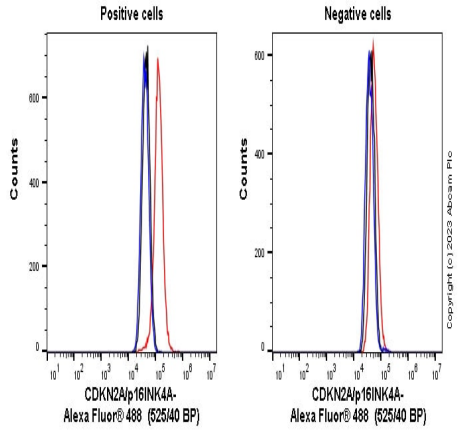
Application	Abreviews	Notes
WB	★★★★★ (3)	1/2000. Predicted molecular weight: 17 kDa. **For unpurified use at 1/1000 - 1/10000.**
IP		1/30. **For unpurified use at 1/10 - 1/100.**
IHC-P	★★★★☆ (2)	1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**. **For unpurified use at 1/250 - 1/500.**
Flow Cyt (Intra)		1/270 - 1/500. For unpurified use at 1/100 - 1/500. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

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



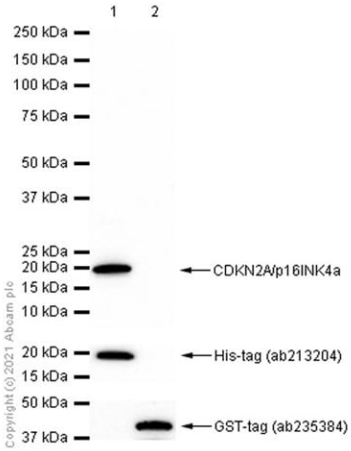
Flow Cytometry (Intracellular) - Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349)

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.



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

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

Lane 1 : Human CDKN2A full-length recombinant protein with His-tag
Lane 2 : Human CDKN2B full-length recombinant protein with GST-tag

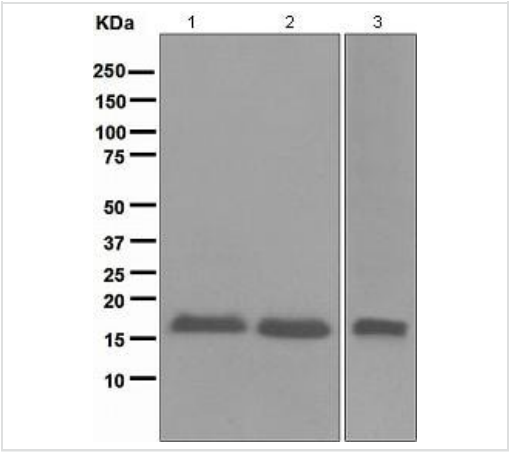
Lysates/proteins at 0.01 µg per lane.

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

Predicted band size: 17 kDa
Observed band size: 17 kDa

Exposure time: 10 seconds

5% NFDM/TBST was used as a blocking and diluting buffer.



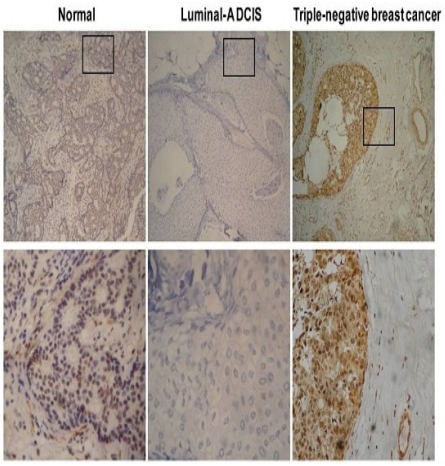
Western blot - Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349)

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

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate
Lane 2 : HEK-293T (Human epithelial cell line from embryonic kidney) cell lysate
Lane 3 : Saos-2 (Human osteosarcoma cell line) cell lysate

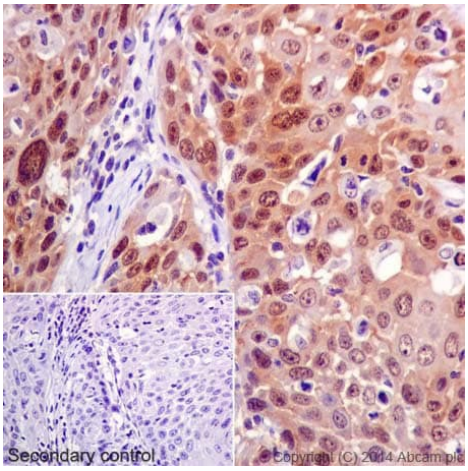
Lysates/proteins at 10 µg per lane.

Predicted band size: 17 kDa



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

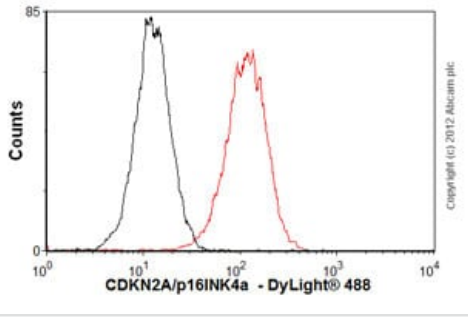
Formalin-fixed, paraffin-embedded human normal breast, luminal-A DCIS (ductal carcinoma *in situ*) and triple negative breast cancer tissues stained for CDKN2A/p16INK4a using ab108349 in immunohistochemical analysis.



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

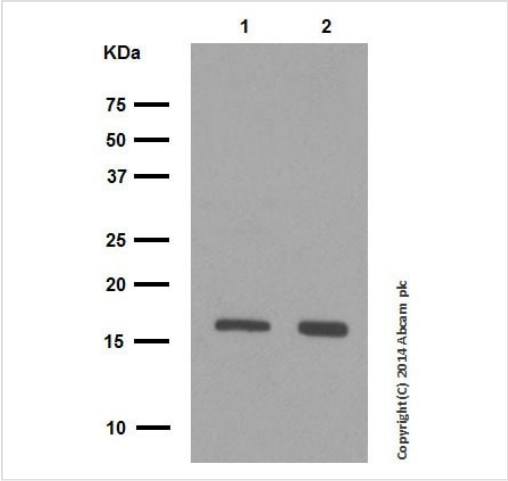
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervix carcinoma tissue labeling CDKN2A/p16INK4a with purified ab108349 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, an HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500).

Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



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

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.



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

All lanes : Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349) at 1/2000 dilution (purified)

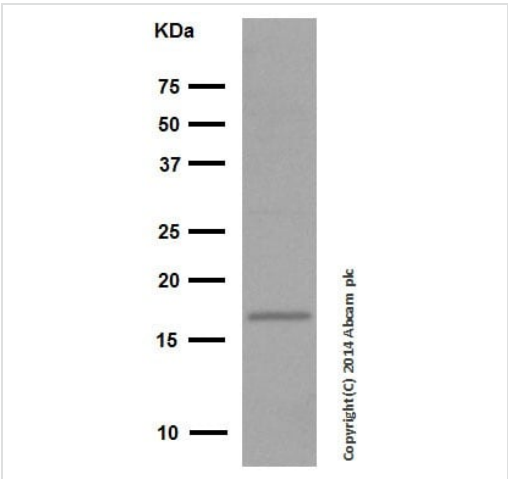
Lane 1 : HEK-293 (Human epithelial cell line from embryonic kidney) cell lysate
Lane 2 : Saos-2 (Human osteosarcoma cell line) cell lysate

Lysates/proteins at 10 µg per lane.

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

Predicted band size: 17 kDa
Observed band size: 17 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



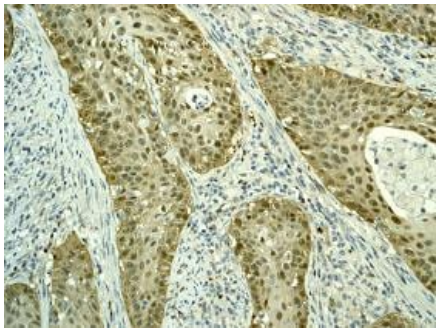
Western blot - Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349)

Anti-CDKN2A/p16INK4a antibody [EPR1473] - C-terminal (ab108349) at 1/2000 dilution (purified) + HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate at 10 µg

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

Predicted band size: 17 kDa
Observed band size: 17 kDa

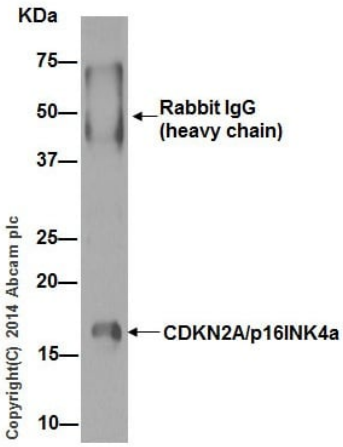
Blocking/Dilution buffer: 5% NFDM/TBST.



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

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.

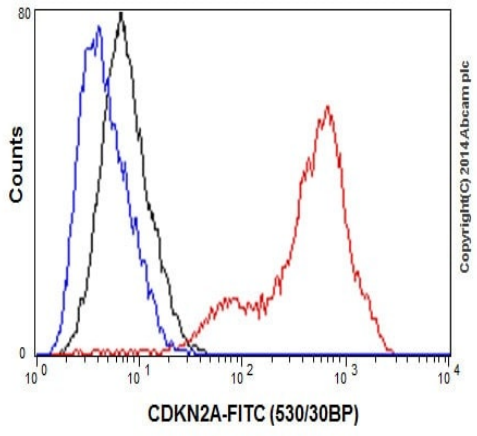
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



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

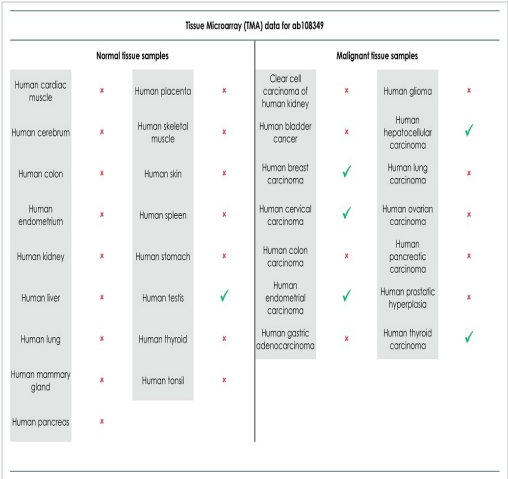
ab108349 (purified) at 1/30 immunoprecipitating CDKN2A/p16INK4a in HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate. For western blotting, a peroxidase-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/1000).

Blocking/Dilution buffer: 5% NFDM/TBST.



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

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)

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] - C-terminal (ab108349)

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