abcam

Product datasheet

Anti-CDKN2A/p16INK4a antibody [EPR20418] ab211542

Recombinant RabMAb

★★★★★ 7 Abreviews 53 References 7 Images

Overview

Product name Anti-CDKN2A/p16INK4a antibody [EPR20418]

Description Rabbit monoclonal [EPR20418] to CDKN2A/p16lNK4a

Host species Rabbit

Specificity Expression levels of the CDKN2A/p16lNK4a protein may vary with sample type. It's 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.

Suitable for: Flow Cyt (Intra), WB, IP, ICC/IF **Tested applications**

Unsuitable for: IHC-P

Species reactivity Reacts with: Mouse

Does not react with: Rat. Human

Immunogen Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: MEF whole cell lysate; His-tagged mouse CDKN2A/p16lNK4a recombinant protein, aa1-

168. ICC/IF: MEF cells. Flow Cyt (intra): MEF cells. IP: MEF whole cell lysate.

General notes 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 long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 0.05% BSA, 40% Glycerol, PBS

Purity Protein A purified

Clonality Monoclonal
Clone number EPR20418

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab211542 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
Flow Cyt (Intra)		1/500.
WB	★★★★☆ (2)	1/2000. Detects a band of approximately 16, 14 kDa (predicted molecular weight: 18 kDa).
IP		1/30.
ICC/IF	★★★★★ (2)	1/100.

Application notes Is unsuitable for IHC-P.

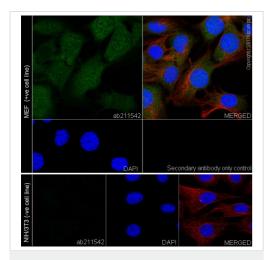
Target

Cellular localization Cytoplasmic and Nuclear

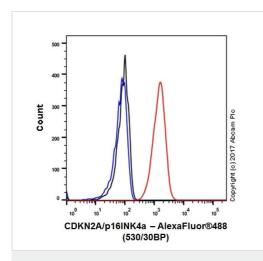
Form There are 4 isoforms produced by alternative splicing. Isoform 1 also known as: p16INK4a;

lsoform 3 also known as: p12; lsoform 4 also known as: p14ARF; p19ARF; ARF.

Images



Immunocytochemistry/ Immunofluorescence - Anti-CDKN2A/p16INK4a antibody [EPR20418] (ab211542)



Flow Cytometry (Intracellular) - Anti-CDKN2A/p16INK4a antibody [EPR20418] (ab211542)

Immunofluorescent analysis of 100% methanol-fixed MEF (mouse embryonic fibroblast cell line) and NIH/3T3 (mouse embryo fibroblast cell line) cells labeling CDKN2A/p16INK4a with ab211542 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1,000 dilution (green). Confocal image showing nuclear and cytoplasmic staining on MEF cell line.

Negative control: NIH/3T3 (PMID: 15210712).

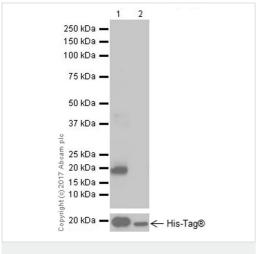
The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) (ab195889) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1,000 dilution.

MEF cells were kindly provided by professor Pinlong Xu, Zhejiang University.

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed MEF (mouse embryonic fibroblast cell line) cell line labeling CDKN2A/p16INK4awith ab211542 at 1/500 dilution (red) compared with a Rabbit lgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (ab150077) at 1/2000 dilution was used as the secondary antibody.

MEF cells were kindly provided by professor Pinlong Xu, Zhejiang University.



Western blot - Anti-CDKN2A/p16lNK4a antibody [EPR20418] (ab211542)

All lanes : Anti-CDKN2A/p16INK4a antibody [EPR20418] (ab211542) at 1/1000 dilution

Lane 1 : His-tagged mouse CDKN2A/p16INK4a recombinant protein

Lane 2 : His-tagged mouse CDKN2B/p15INK4b recombinant protein

Lysates/proteins at 0.01 µg per lane.

Secondary

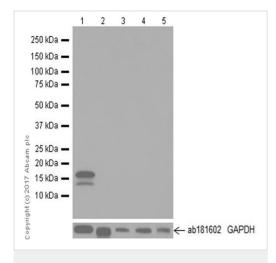
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 18 kDa **Observed band size:** 18 kDa

Exposure time: 1 second

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-CDKN2A/p16lNK4a antibody [EPR20418] (ab211542)

All lanes : Anti-CDKN2A/p16INK4a antibody [EPR20418] (ab211542) at 1/2000 dilution

Lane 1: MEF (mouse embryonic fibroblast cell line) whole cell lysate

Lane 2: NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate

Lane 3: Mouse lung lysate

Lane 4: Mouse spleen lysate

Lane 5: Mouse testis lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$ at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 18 kDa **Observed band size:** 14,16 kDa

Exposure time: 3 seconds

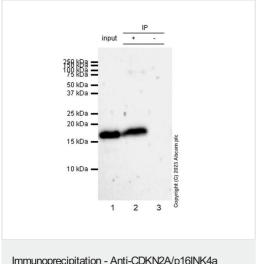
Blocking/Dilution buffer: 5% NFDM/TBST.

Negative control: NIH/3T3 (PMID: 15210712).

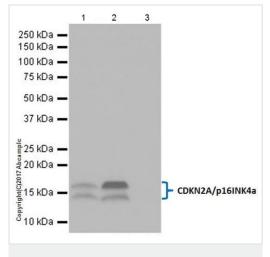
Limited expression in mouse normal tissues has been documented in the literature (PMID: 9244355).

14 kDa band is a proteolytic p16lNK4a lacking C-terminus (PMID: 18053084).

MEF cells are kindly provided by professor Pinlong Xu, Zhejiang University.



Immunoprecipitation - Anti-CDKN2A/p16lNK4a antibody [EPR20418] (ab211542)



Immunoprecipitation - Anti-CDKN2A/p16INK4a antibody [EPR20418] (ab211542)

CDKN2A/p16INK4a was immunoprecipitated from fresh MEF lysates with ab211542 at 1/30 dilution (2ug in 0.35mg lysates). Lysates were made fresh and used immediately to minimize protein degradation. Western blot was performed on the immunoprecipitate using ab211542 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (ab131366) was used at 1/5000 dilution.

Lane 1: MEF (mouse embryo fibroblast) whole cell lysate 10 μ g (IP lysis buffer)

Lane 2: MEF (mouse embryo fibroblast) whole cell lysate (IP lysis buffer)

Lane 3: Rabbit monoclonal $\lg G (\underline{ab172730})$ instead of ab211542 in MEF whole cell lysate (IP lysis buffer)

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

CDKN2A/p16INK4a was immunoprecipitated from 0.35 mg of MEF (mouse embryonic fibroblast cell line) whole cell lysate with ab211542 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab211542 at 1/5000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10.000 dilution

Lane 1: MEF whole cell lysate 10 µg (Input).

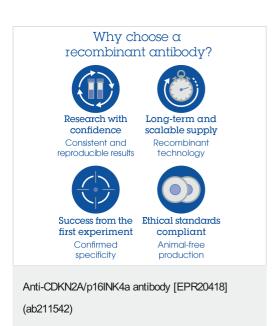
Lane 2: ab211542 IP in MEF whole cell lysate.

Lane 3: Rabbit monoclonal $\lg G$ ($\underline{ab172730}$) instead of ab211542 in MEF whole cell lysate.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 30 seconds.

MEF cells were kindly provided by professor Pinlong Xu, Zhejiang University.



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