## abcam

### Product datasheet

# Anti-CDKN2A/p14ARF antibody [EPR17878] - BSA and Azide free ab224273





RabMAb

1 References 6 图像

#### 概述

产品名称 Anti-CDKN2A/p14ARF抗体[EPR17878] - BSA and Azide free

描述 兔单克隆抗体[EPR17878] to CDKN2A/p14ARF - BSA and Azide free

**宿主** Rabbit

经测试应用 适用于: IP, ICC/IF, WB, Flow Cyt (Intra)

种属反应性 与反应: Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

**阳性**对照 WB: HeLa whole cell lysate transfected with CDKN2A/p14ARF with His-tag; PC-3 and HEK-293

whole cell lysates. ICC/IF: HeLa cells transfected with CDKN2A/p14ARF. Flow Cyt (intra): HeLa cells transfected with CDKN2A/p14ARF-GFP. IP: CDKN2A/p14ARF transfected HeLa whole cell

lysate.

常规说明 ab224273 is the carrier-free version of ab185620.

Our <u>carrier-free</u> 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 <u>conjugation kits</u> 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<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> 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

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#### 性能

形式 Liquid

**存放说明** Shipped at 4°C. Store at +4°C. Do Not Freeze.

**存储溶液** pH: 7.2

Constituent: PBS

**无载体** 是

纯**度** Protein A purified

**克隆** 单克隆

**克隆编号** EPR17878

**同种型** IgG

#### 应用

#### The Abpromise guarantee

Abpromise™承诺保证使用ab224273于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 14 kDa (predicted molecular weight: 14 kDa).
Flow Cyt (Intra)		Use at an assay dependent concentration. <u>ab199376</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

#### 靶标

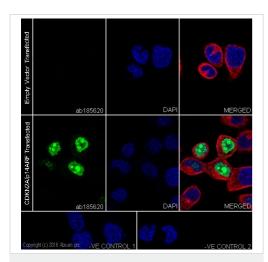
相关性 The gene for CDK2NA generates several transcripts/proteins which differ from each other in their

first exons. Three of these transcripts are generated by alternative splicing (isoform 1 a.k.a p16INK4A, isoform 2 and isoform 3 a.k.a p12), two of which are known to function as inhibitors of CDK4 kinase. One other transcript that is generated from this gene contains an alternate reading frame (ARF), with the first exon located 20kb upstream of the remainder of the gene(isoform 4 a.k.a. p14ARF, p19ARF, ARF). In spite of the structural and some functional differences, all the

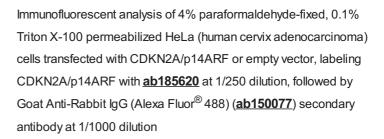
proteins encoded by the CDKN2A gene are involved in cell cycle G1 control.

细胞定位 Cytoplasmic and Nuclear

#### 图片



Immunocytochemistry/ Immunofluorescence - Anti-CDKN2A/p14ARF antibody [EPR17878] - BSA and Azide free (ab224273)



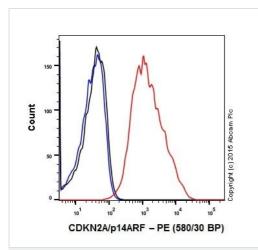
The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) at 1/1000 dilution and Goat Anti-Mouse lgG H&L (Alexa Fluor® 594) preadsorbed (ab150120) at 1/1000 dilution (red).

The negative controls are as follows:-

- -ve control 1: <u>ab185620</u> at 1/250 dilution followed by <u>ab150120</u> at 1/1000 dilution.
- -ve control 2:  $\underline{ab7291}$  at 1/1000 dilution followed by  $\underline{ab150077}$  at 1/1000 dilution.

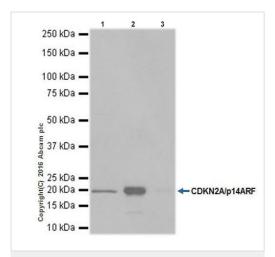
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab185620</u>).



Flow Cytometry (Intracellular) - Anti-CDKN2A/p14ARF antibody [EPR17878] - BSA and Azide free (ab224273)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cell line from cervix adenocarcinoma) cells transfected with CDKN2A/p14ARF-GFP labeling CDKN2A/p14ARF with <a href="mailto:ab185620">ab185620</a> at 1/120 dilution (red) compared with a Rabbit lgG,monoclonal [EPR25A] - Isotype Control (<a href="mailto:ab172730">ab172730</a>) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti Rabbit lgG (PE) at 1/150 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab185620).



Immunoprecipitation - Anti-CDKN2A/p14ARF antibody [EPR17878] - BSA and Azide free (ab224273)

CDKN2A/p14ARF was immunoprecipitated from 1mg of CDKN2A/p14ARF transfected HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate with <u>ab185620</u> at 1/80 dilution.

Western blot was performed from the immunoprecipitate using **ab185620** at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10000 dilution.

Lane 1: CDKN2A/p14ARF transfected HeLa whole cell lysate, 10µg (Input).

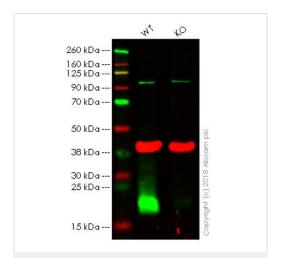
Lane 2: <u>ab185620</u> IP in CDKN2A/p14ARF transfected HeLa whole cell lysate.

Lane 3: Rabbit lgG,monoclonal[EPR25A] - Isotype Control (ab172730) instead of ab185620 in CDKN2A/p14ARF transfected HeLa whole cell lysate.

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

Exposure time: 1 second.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab185620</u>).



Western blot - Anti-CDKN2A/p14ARF antibody [EPR17878] - BSA and Azide free (ab224273)

**All lanes :** Anti-CDKN2A/p14ARF antibody [EPR17878] (ab185620) at 1/1000 dilution

Lane 1: Wild-type HeLa whole cell lysate

Lane 2: CDKN2A knockout HeLa whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 14 kDa

**Lanes 1 - 2:** Merged signal (red and green). Green - <u>ab185620</u> observed at 14 kDa. Red - loading control, <u>ab9484</u>, observed at 37 kDa.

ab185620 was shown to specifically react with CDKN2A/p14ARF in wild-type HeLa cells as signal was lost in CDKN2A knockout cells. Wild-type and CDKN2A knockout samples were subjected to SDS-PAGE. Ab185620 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab185620</u>).

ab185620 DAPI MERGED

Aprilyt (c) 2016 About pic Ve CONTROL 1

Aprilyt (c) 2016 About pic Ve CONTROL 1

Aprilyt (c) 2016 About pic Ve CONTROL 2

Immunocytochemistry/ Immunofluorescence - Anti-CDKN2A/p14ARF antibody [EPR17878] - BSA and Azide free (ab224273)

This ICC data was generated using the same anti-CDKN2A/p14ARF antibody clone [EPR17878] in a different buffer formulation (cat# <u>ab185620</u>).

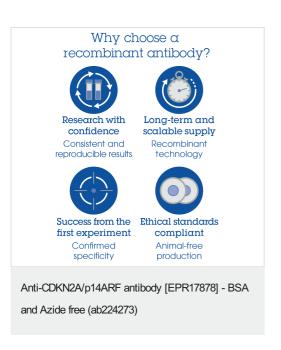
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human cervix adenocarcinoma) cells transfected with CDKN2A/p14ARF or empty vector, labeling CDKN2A/p14ARF with <a href="mailto:ab185620">ab185620</a> at 1/250 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor<sup>®</sup> 488) (<a href="mailto:ab150077">ab150077</a>) secondary antibody at 1/1000 dilution

The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) at 1/1000 dilution and Goat Anti-Mouse lgG H&L (Alexa Fluor® 594) preadsorbed (ab150120) at 1/1000 dilution (red).

The negative controls are as follows:-

- -ve control 1: <u>ab185620</u> at 1/250 dilution followed by <u>ab150120</u> at 1/1000 dilution.
- -ve control 2:  $\underline{ab7291}$  at 1/1000 dilution followed by  $\underline{ab150077}$  at 1/1000 dilution.



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