

### Anti-Cdk4 antibody [EPR17525] ab199728

敲除验证
重组
RabMAb

61 References 11 图像

#### 概述

产品名称	Anti-Cdk4抗体[EPR17525]
描述	兔单克隆抗体[EPR17525] to Cdk4
宿主	Rabbit
经测试应用	适用于: ICC/IF, IP, WB
种属反应性	与反应: Mouse, Rat, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: Hap1, HeLa, C6, PC-12, C2C12, NIH/3T3 whole cell lysate; mouse brain and heart cell lysate. ICC: HeLa and NIH3T3 cells. IP: NIH/3T3 whole cell lysate.
常规说明	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p>

#### 性能

形式	Liquid
存放说明	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.
存储溶液	<p>pH: 7.2</p> <p>Preservative: 0.01% Sodium azide</p> <p>Constituents: PBS, 40% Glycerol, 0.05% BSA</p>
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR17525
同种型	IgG

## 应用

### The Abpromise guarantee

**Abpromise™** 承诺保证使用ab199728于以下的经测试应用

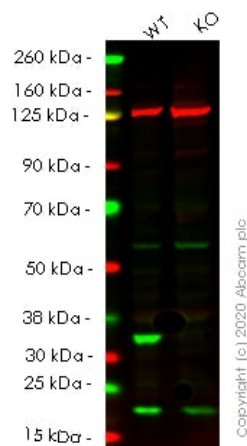
“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ICC/IF		1/250.
IP		1/50.
WB		1/2000. Detects a band of approximately 34 kDa (predicted molecular weight: 34 kDa).

## 靶标

功能	Ser/Thr-kinase component of cyclin D-CDK4 (DC) complexes that phosphorylate and inhibit members of the retinoblastoma (RB) protein family including RB1 and regulate the cell-cycle during G(1)/S transition. Phosphorylation of RB1 allows dissociation of the transcription factor E2F from the RB/E2F complexes and the subsequent transcription of E2F target genes which are responsible for the progression through the G(1) phase. Hypophosphorylates RB1 in early G(1) phase. Cyclin D-CDK4 complexes are major integrators of various mitogenic and antimitogenic signals. Also phosphorylates SMAD3 in a cell-cycle-dependent manner and represses its transcriptional activity. Component of the ternary complex, cyclin D/CDK4/CDKN1B, required for nuclear translocation and activity of the cyclin D-CDK4 complex.
疾病相关	Defects in CDK4 are a cause of susceptibility to cutaneous malignant melanoma type 3 (CMM3) [MIM:609048]. Malignant melanoma is a malignant neoplasm of melanocytes, arising de novo or from a pre-existing benign nevus, which occurs most often in the skin but also may involve other sites.
序列相似性	Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. CDC2/CDKX subfamily. Contains 1 protein kinase domain.
翻译后修饰	Phosphorylation at Thr-172 is required for enzymatic activity. Phosphorylated, in vitro, at this site by CCNH-CDK7, but, in vivo, appears to be phosphorylated by a proline-directed kinase. In the cyclin D-CDK4-CDKN1B complex, this phosphorylation and consequent CDK4 enzyme activity, is dependent on the tyrosine phosphorylation state of CDKN1B. Thus, in proliferating cells, CDK4 within the complex is phosphorylated on Thr-172 in the T-loop. In resting cells, phosphorylation on Thr-172 is prevented by the non-tyrosine-phosphorylated form of CDKN1B.
细胞定位	Cytoplasm. Nucleus. Membrane. Cytoplasmic when non-complexed. Forms a cyclin D-CDK4 complex in the cytoplasm as cells progress through G(1) phase. The complex accumulates on the nuclear membrane and enters the nucleus on transition from G(1) to S phase. Also present in nucleoli and heterochromatin lumps. Colocalizes with RB1 after release into the nucleus.

## 图片



Western blot - Anti-Cdk4 antibody [EPR17525]  
(ab199728)

**All lanes :** Anti-Cdk4 antibody [EPR17525] (ab199728) at 1/2000 dilution

**Lane 1 :** Wild-type HeLa cell lysate

**Lane 2 :** CDK4 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

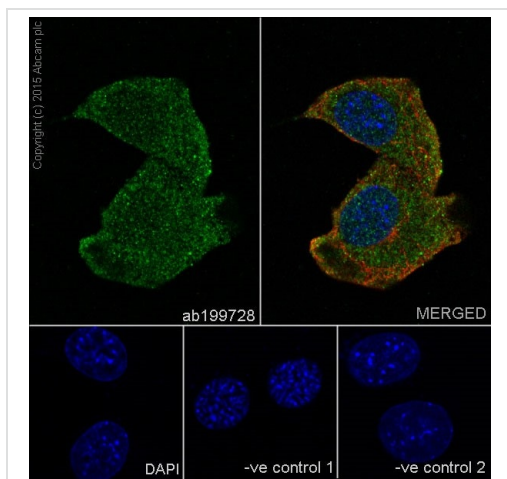
Performed under reducing conditions.

**Predicted band size:** 34 kDa

**Observed band size:** 34 kDa

**Lanes 1- 2:** Merged signal (red and green). Green - ab199728 observed at 34 kDa. Red - Anti-Vinculin antibody [VIN-54] observed at 124 kDa.

ab199728 was shown to react with Cdk4 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab255378** (knockout cell lysate **ab263780**) was used. Wild-type HeLa and CDK4 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab199728 and Anti-Vinculin antibody [VIN-54] overnight at 4°C at a 1 in 2000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-Cdk4 antibody [EPR17525] (ab199728)

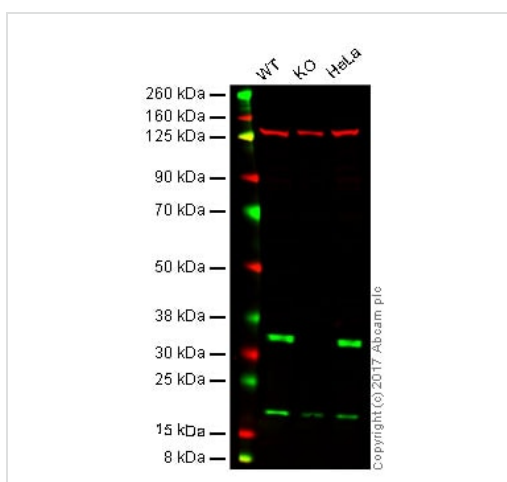
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling Cdk4 with ab199728 at 1/250 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) ([ab150077](#)) secondary antibody at 1/500 dilution (green). Confocal image showing cytoplasmic and nuclear staining on NIH/3T3 cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody- Loading Control ([ab7291](#)) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed ([ab150120](#)) at 1/500 dilution (red).

The negative controls are as follows:

-ve control 1: ab199728 at 1/250 dilution followed by [ab150120](#) at 1/500 dilution.

-ve control 2: [ab7291](#) at 1/1000 dilution followed by [ab150077](#) at 1/500 dilution.



Western blot - Anti-Cdk4 antibody [EPR17525] (ab199728)

**All lanes :** Anti-Cdk4 antibody [EPR17525] (ab199728) at 1/1000 dilution

**Lane 1 :** Wild-type HAP1 whole cell lysate

**Lane 2 :** Cdk4 knockout HAP1 whole cell lysate

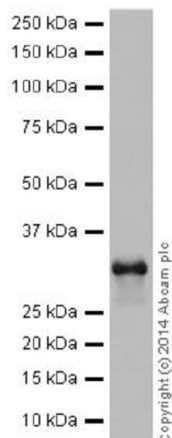
**Lane 3 :** Wild-type HeLa whole cell lysate

Lysates/proteins at 20 µg per lane.

**Predicted band size:** 34 kDa

**Lanes 1 - 3:** Merged signal (red and green). Green - ab199728 observed at 34 kDa. Red - loading control, [ab18058](#), observed at 130 kDa.

ab199728 was shown to specifically recognize Cdk4 in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when Cdk4 knockout samples were examined. Wild-type and Cdk4 knockout samples were subjected to SDS-PAGE. Ab199728 and [ab18058](#) (Mouse anti Vinculin 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.



Western blot - Anti-Cdk4 antibody [EPR17525] (ab199728)

Anti-Cdk4 antibody [EPR17525] (ab199728) at 1/2000 dilution + NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate at 10 µg

### Secondary

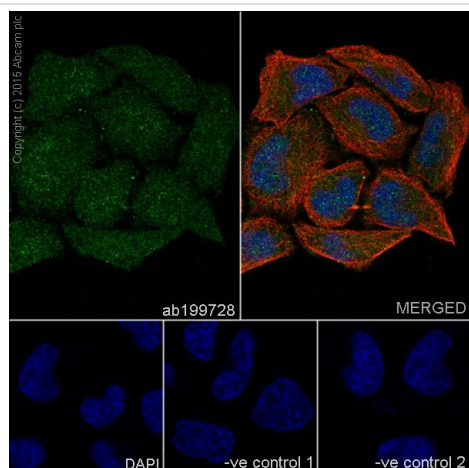
Goat Anti-Rabbit IgG H&L Peroxidase conjugated at 1/1000 dilution

Developed using the ECL technique.

**Predicted band size:** 34 kDa

**Exposure time:** 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-Cdk4 antibody [EPR17525] (ab199728)

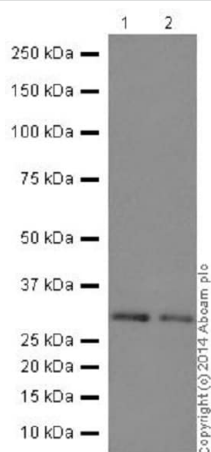
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Cdk4 with ab199728 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H & L (Alexa Fluor® 488) ([ab150077](#)) secondary antibody at 1/500 dilution (green). Confocal image showing cytoplasmic, nuclear and membrane staining on HeLa cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody - Loading Control ([ab7291](#)) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed ([ab150120](#)) at 1/500 dilution (red).

The negative controls are as follows:

-ve control 1: ab199728 at 1/250 dilution followed by [ab150120](#) at 1/500 dilution.

-ve control 2: [ab7291](#) at 1/1000 dilution followed by [ab150077](#) at 1/500 dilution.



Western blot - Anti-Cdk4 antibody [EPR17525]  
(ab199728)

**All lanes :** Anti-Cdk4 antibody [EPR17525] (ab199728) at 1/10000 dilution

**Lane 1 :** Mouse brain whole cell lysate

**Lane 2 :** Mouse heart whole cell lysate

Lysates/proteins at 10 µg per lane.

#### Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L Peroxidase conjugated at 1/1000 dilution

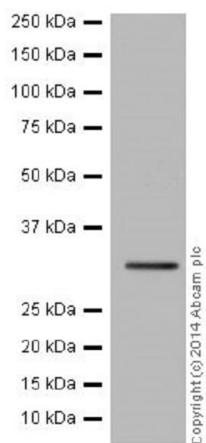
Developed using the ECL technique.

**Predicted band size:** 34 kDa

**Observed band size:** 34 kDa

**Exposure time:** 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Cdk4 antibody [EPR17525]  
(ab199728)

Anti-Cdk4 antibody [EPR17525] (ab199728) at 1/20000 dilution +  
C2C12 (Mouse myoblast cell line) whole cell lysate at 10 µg

### Secondary

Goat Anti-Rabbit IgG H&L, Peroxidase conjugated at 1/1000  
dilution

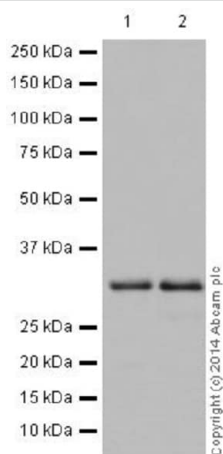
Developed using the ECL technique.

**Predicted band size:** 34 kDa

**Observed band size:** 34 kDa

**Exposure time:** 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Cdk4 antibody [EPR17525]  
(ab199728)

**All lanes :** Anti-Cdk4 antibody [EPR17525] (ab199728) at 1/2000  
dilution

**Lane 1 :** C6 (Rat glial tumor cell line)

**Lane 2 :** PC-12 (Rat adrenal gland pheochromocytoma cell line)

Lysates/proteins at 10 µg per lane.

### Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L, Peroxidase conjugated at  
1/1000 dilution

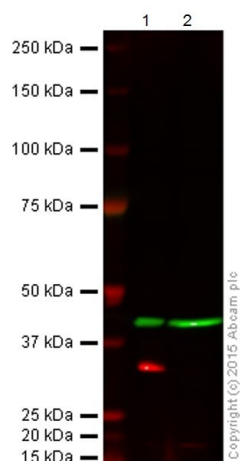
Developed using the ECL technique.

**Predicted band size:** 34 kDa

**Observed band size:** 34 kDa

**Exposure time:** 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Cdk4 antibody [EPR17525]  
(ab199728)

**All lanes :** Anti-Cdk4 antibody [EPR17525] (ab199728) at 1/1000 dilution

**Lane 1 :** WT HAP1 cell lysate

**Lane 2 :** CDK4 knockout HAP1 cell lysate

Lysates/proteins at 20 µg per lane.

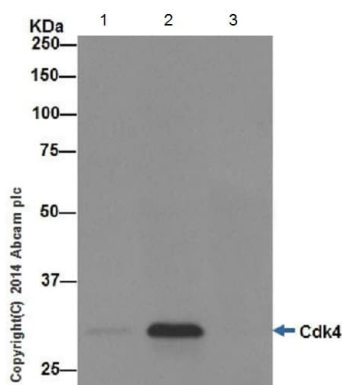
### Secondary

**All lanes :** Goat anti-Rabbit IgG H&L (IRDye® 800CW) at 1/10000 dilution

**Predicted band size:** 34 kDa

**Observed band size:** 34 kDa

ab199728 was shown to specifically react with CDK4 when CDK4 knockout samples were used. Wild-type and CDK4 knockout samples were subjected to SDS-PAGE. ab199728 and [ab82226](#) (loading control to beta actin) were both diluted at 1/1000 and incubated overnight at 4°C. Blots were developed with goat anti-rabbit IgG (H + L) and goat anti-mouse IgG (H + L) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging.



Immunoprecipitation - Anti-Cdk4 antibody  
[EPR17525] (ab199728)

Cdk4 was immunoprecipitated from 1mg NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate with ab199728 at 1/50 dilution. Western blot was performed from the immunoprecipitate using ab199728 at 1/1000 dilution. Anti-Rabbit IgG (HRP) specific to the non-reduced form of IgG was used as secondary antibody at 1/1500 dilution.

Lane 1: NIH/3T3 whole cell lysate, 10 (Input).

Lane 2: ab199728 IP in NIH/3T3 whole cell lysate.

Lane 3: Rabbit IgG, monoclonal [EPR25A]- Isotype Control ([ab172730](#)) instead of ab199728 in NIH/3T3 whole cell lysate.

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

Exposure time: 10 seconds



### Why choose a recombinant antibody?



**Research with confidence**  
Consistent and reproducible results



**Long-term and scalable supply**  
Recombinant technology



**Success from the first experiment**  
Confirmed specificity



**Ethical standards compliant**  
Animal-free production

Anti-Cdk4 antibody [EPR17525] (ab199728)

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