abcam

Product datasheet

Anti-Cdk4 antibody [EPR17525] ab199728

KO VALIDATED Recombinant RabMAb

61 References 11 Images

Overview

Product name	Anti-Cdk4 antibody [EPR17525]
Description	Rabbit monoclonal [EPR17525] to Cdk4
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, IP, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	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.
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: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR17525
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab199728 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
ICC/IF		1/250.
IP		1/50.
WB		1/2000. Detects a band of approximately 34 kDa (predicted molecular weight: 34 kDa).

Target

Function 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 mitogenenic 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.

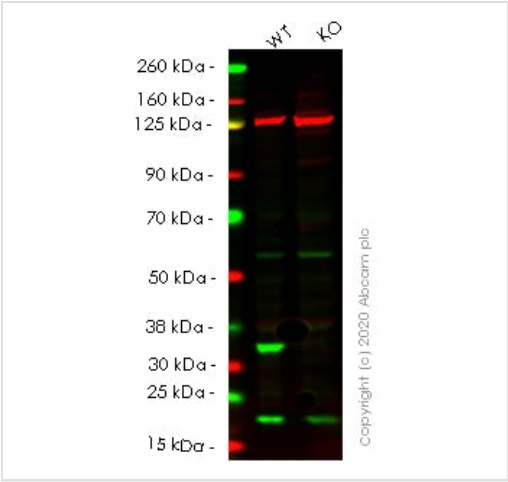
Involvement in disease Defects in CDK4 are a cause of susceptibility to cutaneous malignant melanoma type 3 (CMM3) [MIM:609048]. Malignant melanoma is a malignant neoplasm of melanocytes, arising de novo or from a pre-existing benign nevus, which occurs most often in the skin but also may involve other sites.

Sequence similarities Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. CDC2/CDKX subfamily.
Contains 1 protein kinase domain.

Post-translational modifications 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.

Cellular localization 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.

Images



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

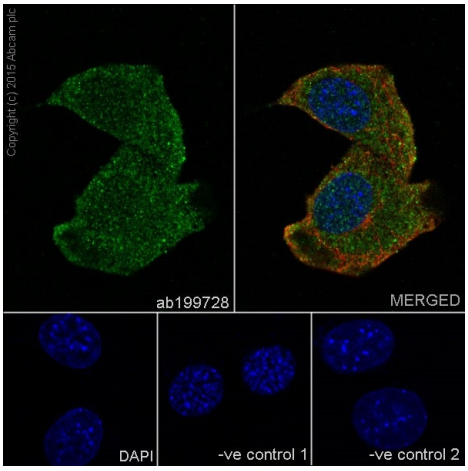
Lysates/proteins at 20 µg per lane.

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)

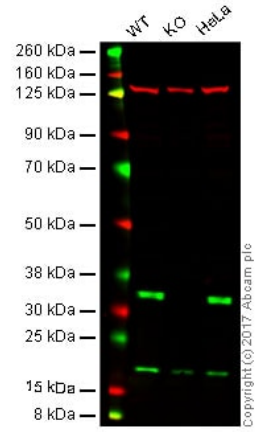
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling Cdk4 with ab199728 at 1/250 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/500 dilution (green). 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

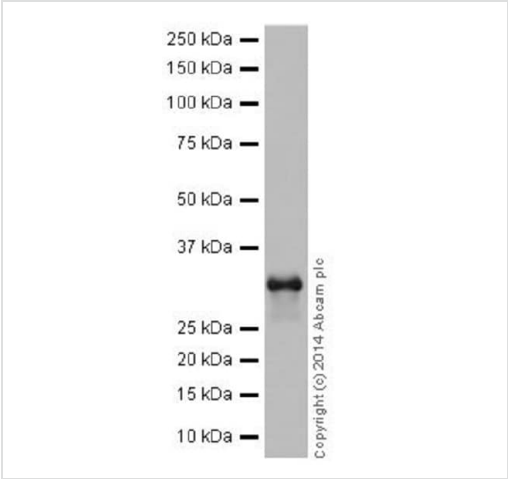
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : Cdk4 knockout HAP1 whole cell lysate
Lane 3 : Wild-type HeLa whole cell lysate

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

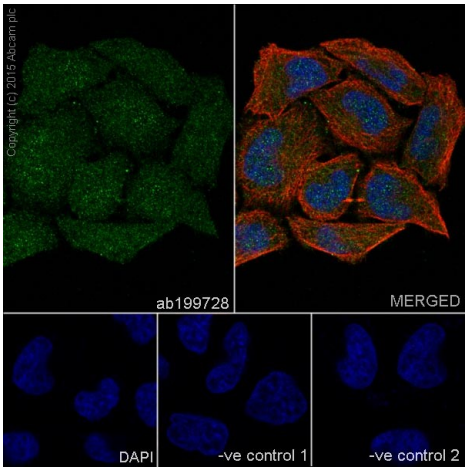
Goat Anti-Rabbit IgG H&L Peroxidase conjugated at 1/1000 dilution

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)

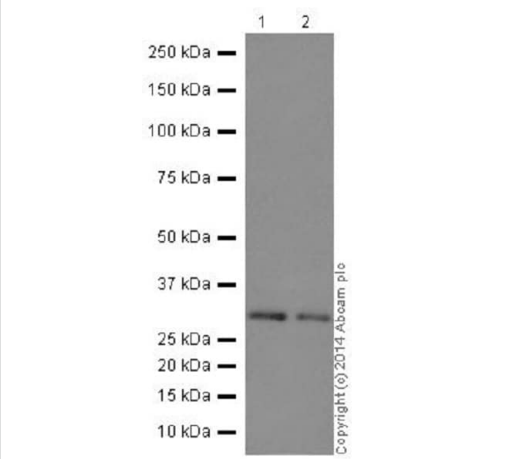
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Cdk4 with ab199728 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H & L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/500 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.

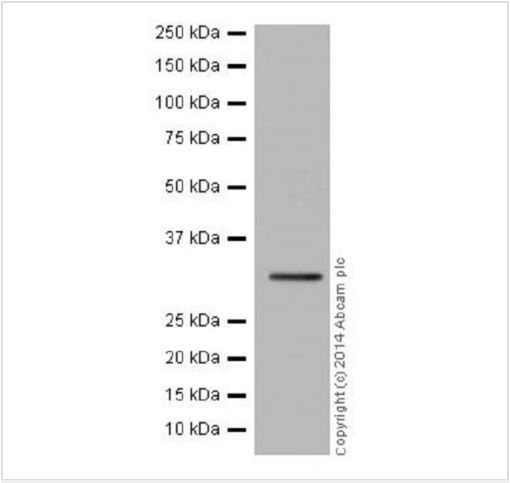
Secondary
All lanes : Goat Anti-Rabbit IgG H&L Peroxidase conjugated at 1/1000 dilution

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

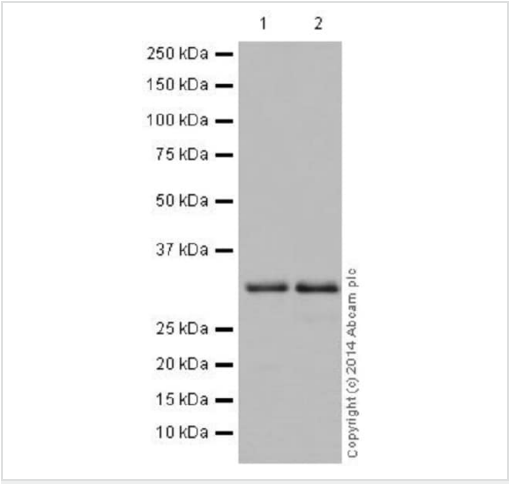
Secondary
Goat Anti-Rabbit IgG H&L, Peroxidase conjugated at 1/1000 dilution

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.

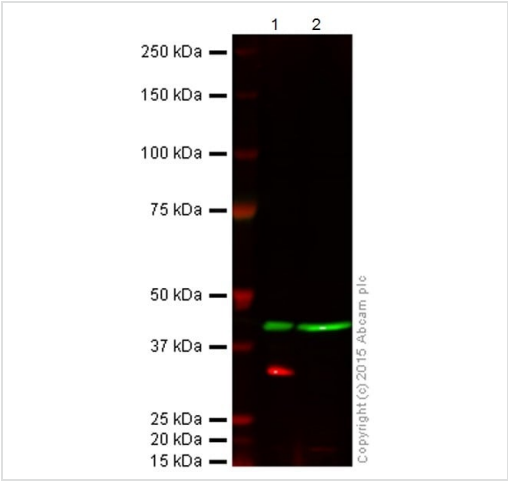
Secondary
All lanes : Goat Anti-Rabbit IgG H&L, Peroxidase conjugated at 1/1000 dilution

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

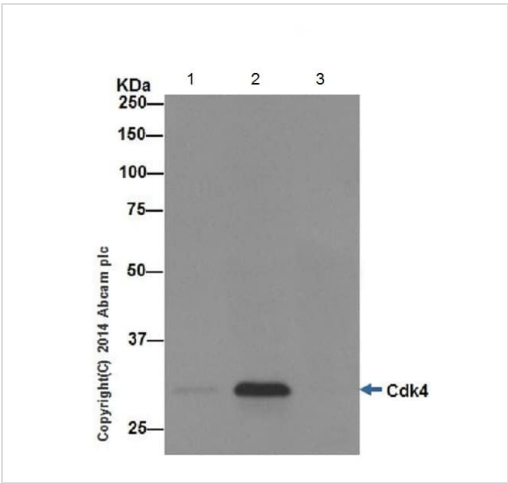
Lane 1 : WT HAP1 cell lysate
Lane 2 : CDK4 knockout HAP1 cell lysate

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 **ab8226** (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.

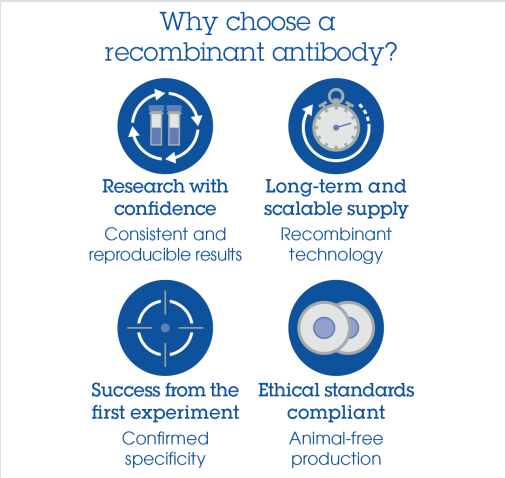
Lane 1: NIH/3T3 whole cell lysate, 10 (Input).

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



Anti-Cdk4 antibody [EPR17525] (ab199728)

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