

Anti-Cdk4 antibody [EPR17525] ab199728

KO 評価済 リコンビナント RabMAb

61 References 画像数 11

製品の概要

製品名	Anti-Cdk4 antibody [EPR17525]
製品の詳細	Rabbit monoclonal [EPR17525] to Cdk4
由来種	Rabbit
アプリケーション	適用あり: ICC/IF, IP, WB
種交差性	交差種: Mouse, Rat, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	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.
特記事項	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

製品の特性

製品の状態	Liquid
保存方法	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.
バッファー	<p>pH: 7.2</p> <p>Preservative: 0.01% Sodium azide</p> <p>Constituents: PBS, 40% Glycerol, 0.05% BSA</p>
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR17525
アイソタイプ	IgG

アプリケーション

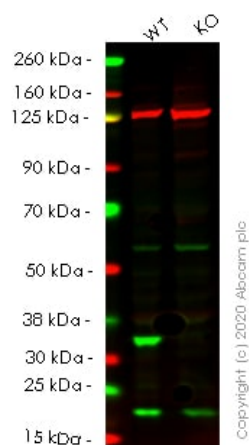
The Abpromise guarantee Abpromise保証は、 次のテスト済みアプリケーションにおけるab199728の使用に適用されます
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
ICC/IF		1/250.
IP		1/50.
WB		1/2000. Detects a band of approximately 34 kDa (predicted molecular weight: 34 kDa).

ターゲット情報

機能	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.
関連疾患	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.
配列類似性	Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. CDC2/CDKX subfamily. Contains 1 protein kinase domain.
翻訳後修飾	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.
細胞内局在	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.

画像



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.

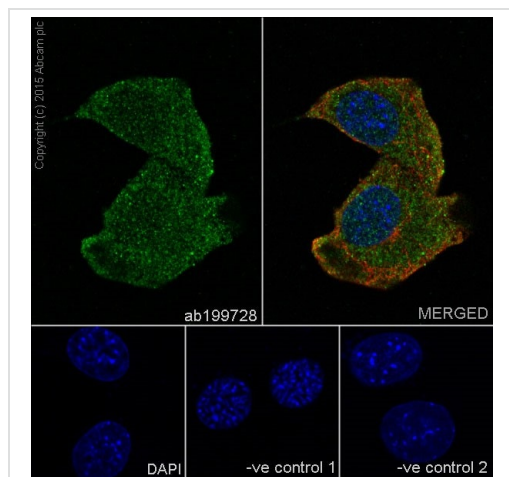
Performed under reducing conditions.

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)

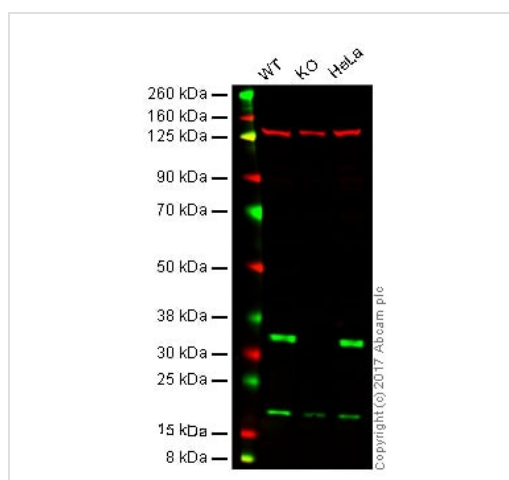
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling Cdk4 with ab199728 at 1/250 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) ([ab150077](#)) secondary antibody at 1/500 dilution (green).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

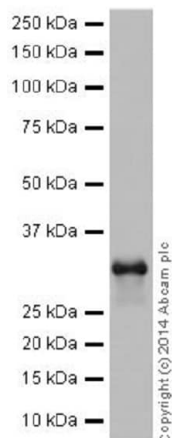
Lane 3 : Wild-type HeLa whole cell lysate

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

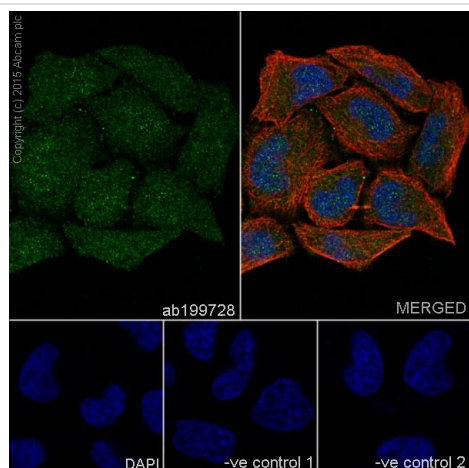
Goat Anti-Rabbit IgG H&L Peroxidase conjugated at 1/1000 dilution

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)

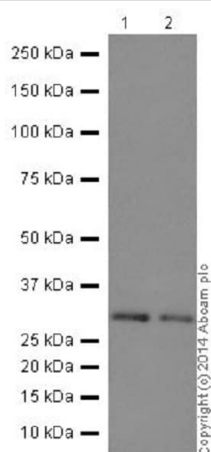
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Cdk4 with ab199728 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H & L (Alexa Fluor® 488) ([ab150077](#)) secondary antibody at 1/500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

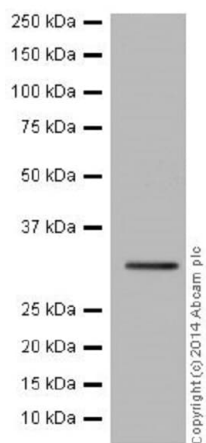
Developed using the ECL technique.

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

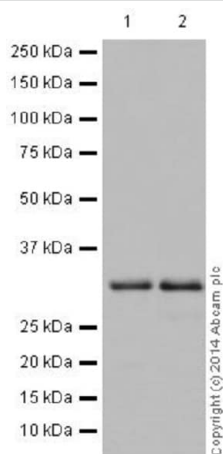
Developed using the ECL technique.

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

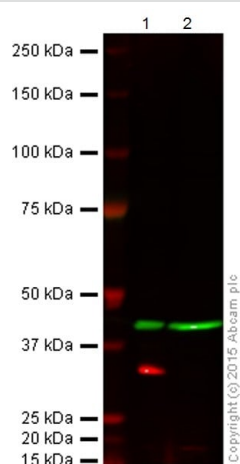
Developed using the ECL technique.

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.

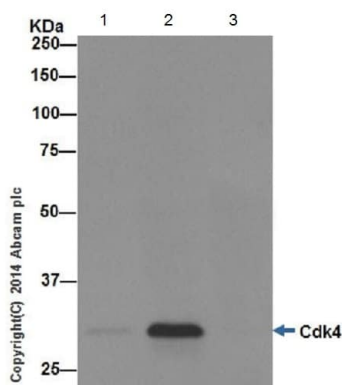
Secondary

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?



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Recombinant technology



Success from the first experiment
Confirmed specificity



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Anti-Cdk4 antibody [EPR17525] (ab199728)

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