

Product datasheet

Anti-Cdk4 antibody [EPR2513Y] ab68266

KO VALIDATED Recombinant RabMAB

8 References 7 Images

Overview

Product name	Anti-Cdk4 antibody [EPR2513Y]
Description	Rabbit monoclonal [EPR2513Y] to Cdk4
Host species	Rabbit
Tested applications	Suitable for: ICC/IF, WB, IP, Flow Cyt Unsuitable for: IHC-P
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide corresponding to Human Cdk4 aa 1-100 (N terminal).
Positive control	WB: HeLa, MCF-7, Ramos and K562 cell lysates. Rat lung lysates, Mouse lung lysates, ICC/IF: Wildtype HAP1 cells, MCF7 cells. FC: A549 cell IP: K562 whole cell lysate
General notes	<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> <p>We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.</p> <p>This product is a recombinant rabbit monoclonal antibody.</p>

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: 7.40 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.5% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR2513Y

Isotype

IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab68266** 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		1/100. For unpurified use at 1/250
WB		1/500 - 1/1000. Detects a band of approximately 34 kDa (predicted molecular weight: 34 kDa).
IP		1/30.
Flow Cyt		1/500. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For unpurified use at 1/60.00000

Application notes

Is unsuitable for IHC-P.

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 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.

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

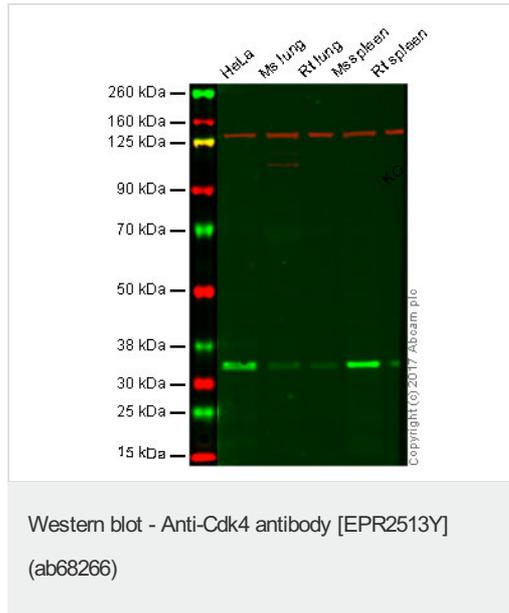
Phosphorylation at Thr-172 is required for enzymatic activity. Phosphorylated, in vitro, at this site by CCNH-CDK7, but, in vivo, appears to be phosphorylated by a proline-directed kinase. In the cyclin D-CDK4-CDKN1B complex, this phosphorylation and consequent CDK4 enzyme activity, is dependent on the 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



All lanes : Anti-Cdk4 antibody [EPR2513Y] (ab68266) at 1/1000 dilution (Unpurified)

Lane 1 : HeLa whole cell lysate

Lane 2 : Mouse lung tissue lysate

Lane 3 : Rat lung tissue lysate

Lane 4 : Mouse spleen tissue lysate

Lane 5 : Rat spleen tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

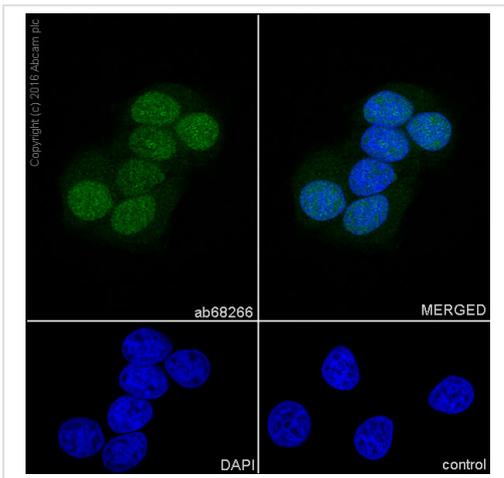
Performed under reducing conditions.

Predicted band size: 34 kDa

Observed band size: 34 kDa

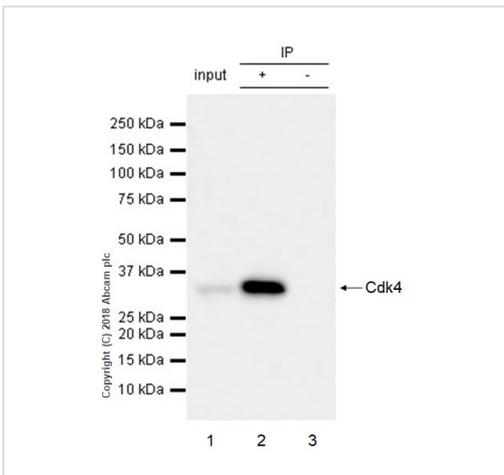
Lanes 1 - 5: Merged signal (red and green). Green - ab68266 observed at 34 kDa. Red - loading control, ab18058, observed at 130 kDa.

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using Licor blocking buffer before being incubated with ab68266 and ab18058 (loading control) overnight at 4°C. Antibody binding was detected using Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at a 1:10000 dilution for 1hr at room temperature and then imaged.



Immunocytochemistry/ Immunofluorescence - Anti-Cdk4 antibody [EPR2513Y] (ab68266)

Immunocytochemistry/ Immunofluorescence analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling Cdk4 with Purified ab68266 at 1:100 (5.4 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with None. Goat anti rabbit IgG (Alexa Fluor® 488, [ab150077](#)) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Immunoprecipitation - Anti-Cdk4 antibody [EPR2513Y] (ab68266)

ab68266 (purified) at 1:30 dilution (2µg) immunoprecipitating Cdk4 in K562 whole cell lysate.

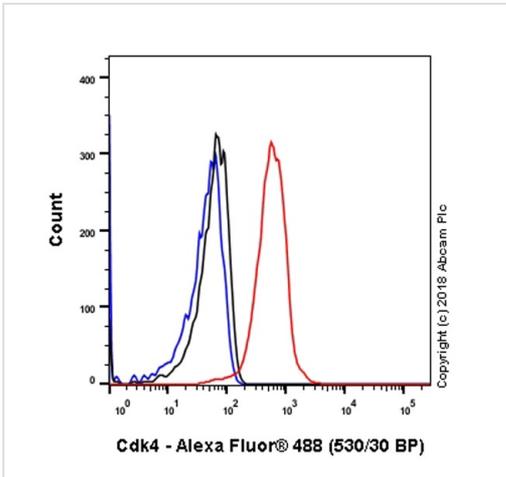
Lane 1 (input): K562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysate 10µg

Lane 2 (+): ab68266 & K562 whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG ([ab172730](#)) instead of ab68266 in K562 whole cell lysate

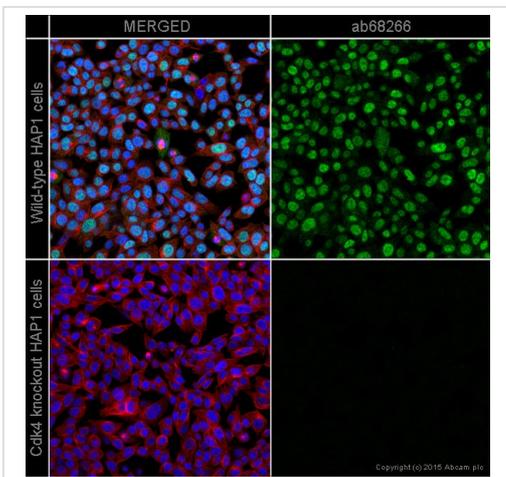
For western blotting, VeriBlot for IP Detection Reagent (HRP) ([ab131366](#)) was used for detection at 1:1000 dilution.

Blocking and diluting buffer: 5% NFDm/TBST.



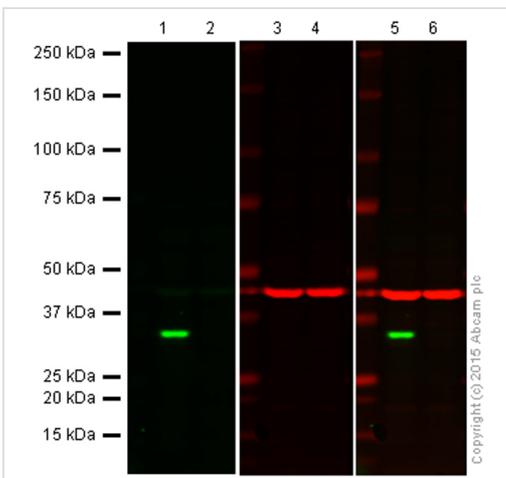
Flow Cytometry - Anti-Cdk4 antibody [EPR2513Y] (ab68266)

Flow Cytometry analysis of A549 (Human lung carcinoma epithelial cell) cells labeling Cdk4 with purified ab68266 at 1:50 dilution (1 µg/ml) (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1:2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunocytochemistry/ Immunofluorescence - Anti-Cdk4 antibody [EPR2513Y] (ab68266)

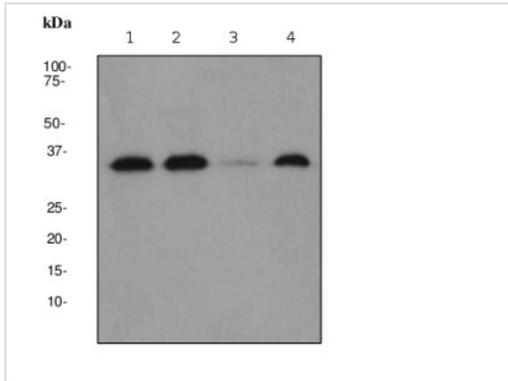
Unpurified ab68266 staining Cdk4 in wild-type HAP1 cells (top panel) and Cdk4 knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab68266 at 1/250 dilution and ab195889 at 1/250 dilution (shown in pseudo colour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.



Western blot - Anti-Cdk4 antibody [EPR2513Y] (ab68266)

Lanes 1, 3 and 5: Wild-type HAP1 cell lysate (20 µg)
Lanes 2, 4 and 6: CDK4 knockout HAP1 cell lysate (20 µg)
Lanes 1 and 2: Green signal from target - ab68266 observed at 34 kDa
Lanes 3 and 4: Red signal from loading control - ab8226 observed at 42 kDa
Lanes 5 and 6: Merged (red and green) signal
 Unpurified ab68266 was shown to specifically react with CDK4 when CDK4 knockout samples were used. Wild-type and CDK4 knockout samples were subjected to SDS-PAGE. ab68266 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 (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10000 dilution for 1 h at

room temperature before imaging.



Western blot - Anti-Cdk4 antibody [EPR2513Y]
(ab68266)

All lanes : Anti-Cdk4 antibody [EPR2513Y] (ab68266) at 1/500 dilution (Unpurified)

Lane 1 : HeLa cell lysate

Lane 2 : MCF7 cell lysate

Lane 3 : Romas cell lysate

Lane 4 : K452 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : HRP conjugated Goat anti-rabbit at 1/2000 dilution

Predicted band size: 34 kDa

Observed band size: 34 kDa

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