abcam

Product datasheet

Anti-Cdk4 antibody [EPR4513-32-7] ab108357

KO VALIDATED Recombinant RabMAb

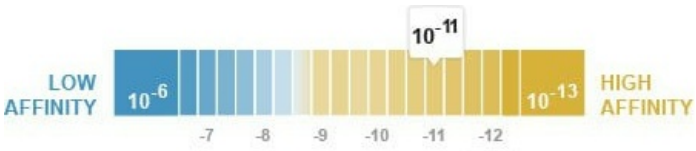
★★★★★ 1 Abreviews 140 References 12 Images

Overview

Product name	Anti-Cdk4 antibody [EPR4513-32-7]
Description	Rabbit monoclonal [EPR4513-32-7] to Cdk4
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, Flow Cyt (Intra), ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1, HeLa, MCF7, K562, and Ramos cell lysates. IHC-P: Human urothelial carcinoma and cervix carcinoma tissues. ICC/IF: Wild type HAP1 cells; MCF7 cells. Flow Cyt (intra): MCF-7 cells.
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**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

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. Stable for 12 months at -20°C.
Dissociation constant (K_D)	$K_D = 1.86 \times 10^{-11}$ M



Learn more about K_D

Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR4513-32-7
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab108357 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

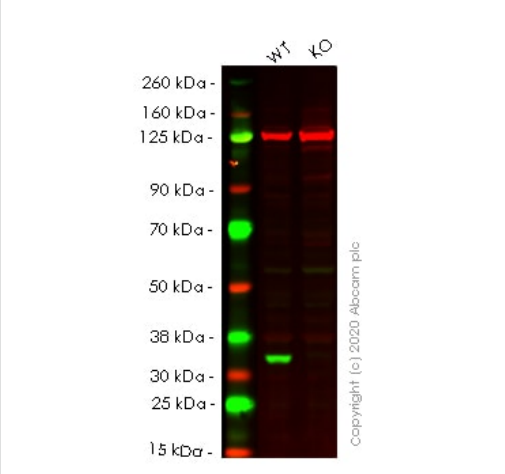
Application	Abreviews	Notes
WB	★★★★★ (1)	1/1000 - 1/10000. Detects a band of approximately 34 kDa (predicted molecular weight: 34 kDa).
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
Flow Cyt (Intra)		1/10 - 1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/500.

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 [EPR4513-32-7] (ab108357)

All lanes : Anti-Cdk4 antibody [EPR4513-32-7] (ab108357) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : CDK4 knockout HeLa cell lysate

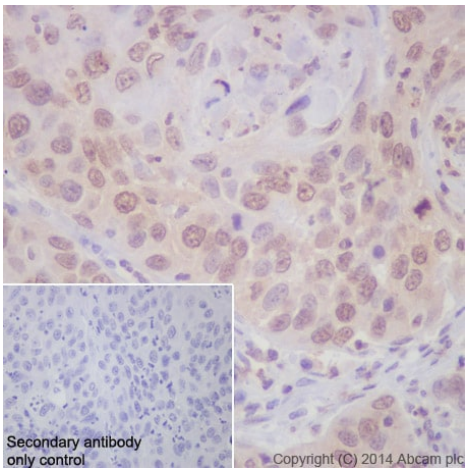
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 34 kDa
Observed band size: 34 kDa

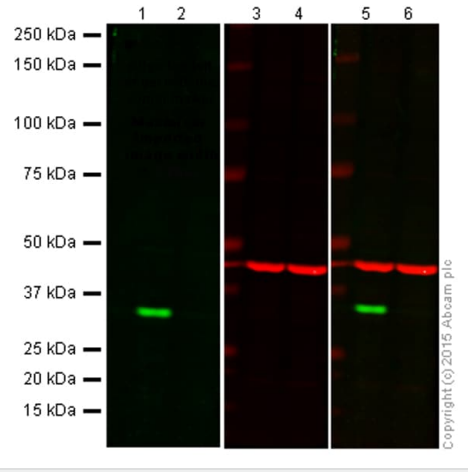
Lanes 1- 2: Merged signal (red and green). Green - ab108357 observed at 34 kDa. Red - Anti-Vinculin antibody [VIN-54] observed at 124 kDa.

ab108357 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. ab108357 and Anti-Vinculin antibody [VIN-54] overnight at 4°C at a 1 in 1000 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cdk4 antibody [EPR4513-32-7] (ab108357)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervix carcinoma tissue labeling Cdk4 with purified ab108357 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer (pH 9). **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L), was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Western blot - Anti-Cdk4 antibody [EPR4513-32-7] (ab108357)

Lanes 1-2 : Anti-Cdk4 antibody [EPR4513-32-7] (ab108357) at 1/1000 dilution
Lanes 3-4 : Anti-beta Actin antibody [mAbcam 8226] - Loading Control (**ab8226**) at 1/1000 dilution

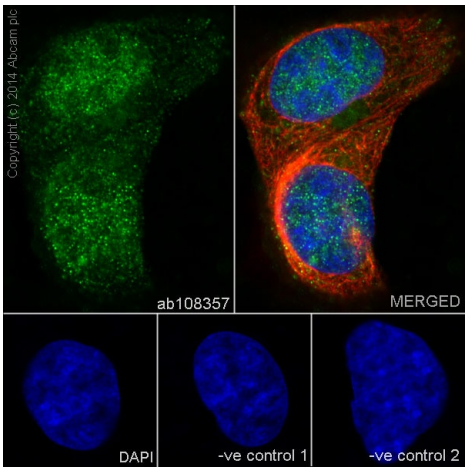
Lanes 1 & 3 & 5 : Wild-type HAP1 cell lysate
Lanes 2 & 4 & 6 : CDK4 knockout HAP1 cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 34 kDa

Lanes 5 and 6: Merged signal (red and green). Green - ab108357 observed at 34kDa. Red - loading control to beta actin observed at 40kDa.

ab108357 was shown to specifically react with CDK4 in wild-type HAP1 cells. No band was observed when CDK4 knockout samples were examined. Wild-type and CDK4 knockout samples were subjected to SDS-PAGE. ab108357 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 1/10,000 dilution for 1 hour at room temperature before imaging.

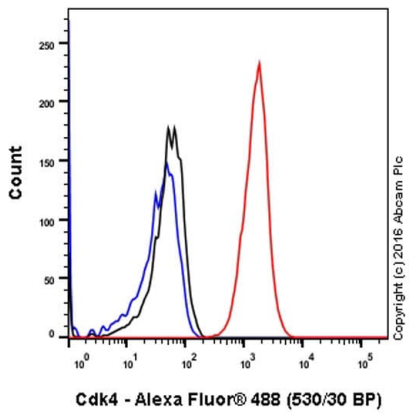


Immunocytochemistry/ Immunofluorescence - Anti-Cdk4 antibody [EPR4513-32-7] (ab108357)

Immunocytochemical analysis of MCF7 (human breast adenocarcinoma cell line) cells, labeling Cdk4 with purified ab108357 at 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500), was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse anti-tubulin (1/1000) and **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500) were also used.

Control 1: primary antibody (1/100) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).

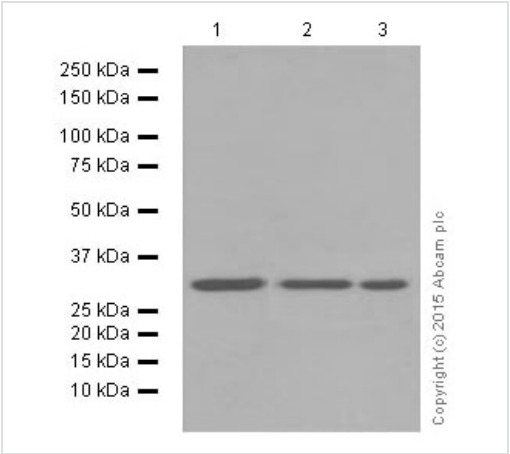


Flow Cytometry (Intracellular) - Anti-Cdk4 antibody [EPR4513-32-7] (ab108357)

ab108357 staining CDK4in the human cell line MCF7 (human breast adenocarcinoma cell line) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde, permeabilized with 90% methanol and the sample was incubated with the primary antibody at a dilution of 1/20. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/2000 was used as the secondary antibody.

Isoytype control: Rabbit monoclonal IgG (Black)

Unlabeled control: Cell without incubation with primary antibody and secondary antibody (Blue)



Western blot - Anti-Cdk4 antibody [EPR4513-32-7] (ab108357)

All lanes : Anti-Cdk4 antibody [EPR4513-32-7] (ab108357) at 1/10000 dilution (purified)

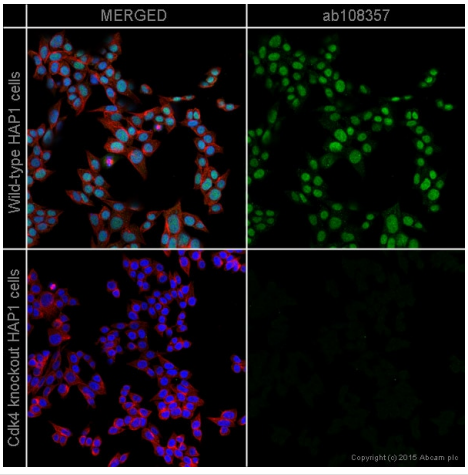
Lane 1 : HeLa (human epithelial cell line from cervix adenocarcinoma) cell lysate
Lane 2 : K562 (human chronic myelogenous leukemia cell line from bone marrow) cell lysate
Lane 3 : Ramos (human Burkitt's lymphoma cell line) cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

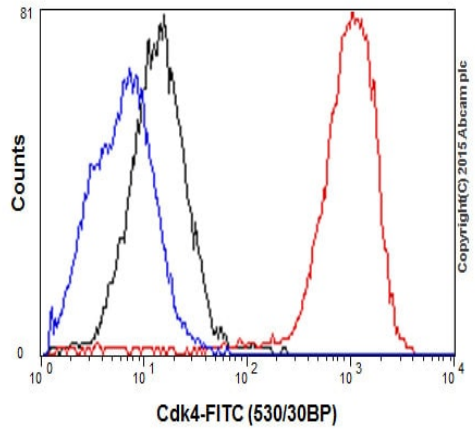
Predicted band size: 34 kDa
Observed band size: 34 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



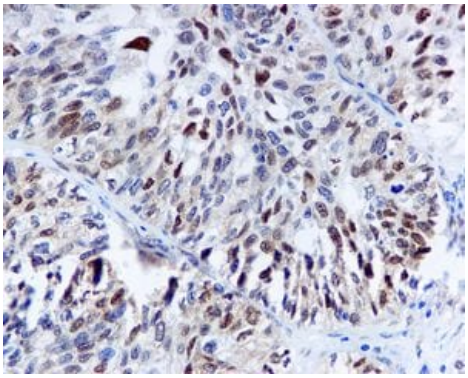
Immunocytochemistry/ Immunofluorescence - Anti-Cdk4 antibody [EPR4513-32-7] (ab108357)

ab108357 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 ab108357 at 1/500 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.



Flow Cytometry (Intracellular) - Anti-Cdk4 antibody [EPR4513-32-7] (ab108357)

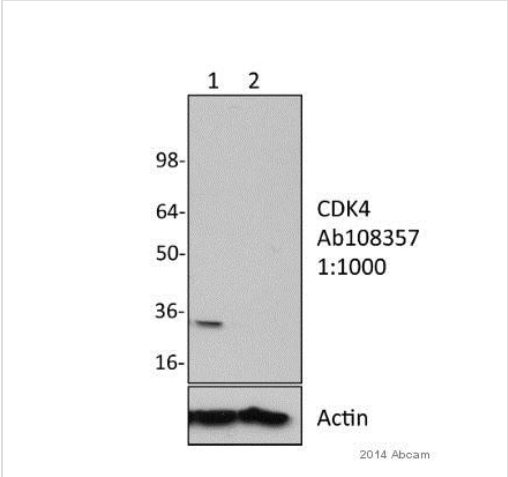
Intracellular Flow Cytometry analysis of MCF7 (human breast adenocarcinoma cell line) cells labelling Cdk4 with purified ab108357 at 1/40 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cdk4 antibody [EPR4513-32-7] (ab108357)

Immunohistochemical analysis of formalin/PFA-fixed paraffin-embedded human urothelial carcinoma tissue labelling Cdk4 with unpurified ab108357 at a dilution of 1/100.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Western blot - Anti-Cdk4 antibody [EPR4513-32-7] (ab108357)

This image is courtesy of an Abreview submitted by Sonia Rocha

All lanes : Anti-Cdk4 antibody [EPR4513-32-7] (ab108357) at 1/1000 dilution (unpurified)

Lane 1 : Human osteosarcoma whole cell lysate - control, non-targeting siRNA
Lane 2 : Human osteosarcoma whole cell lysate - siRNA for CDK4

Lysates/proteins at 20 µg per lane.

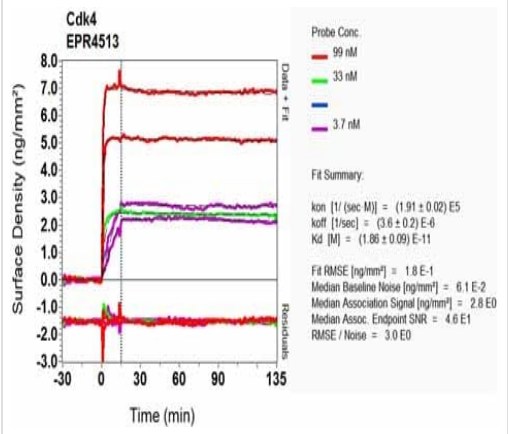
Secondary
All lanes : HRP-conjugated goat anti-rabbit IgG polyclonal at 1/2000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 34 kDa
Observed band size: 34 kDa

Exposure time: 5 seconds

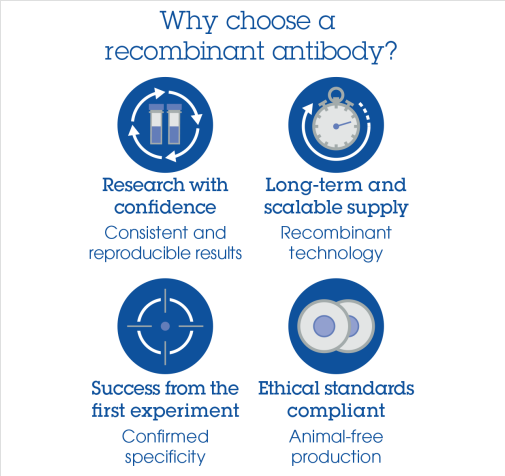


OI-RD Scanning - Anti-Cdk4 antibody [EPR4513-32-7] (ab108357)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D



Anti-Cdk4 antibody [EPR4513-32-7] (ab108357)

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