abcam

Product datasheet

Anti-Cdk4 antibody [EPR4513-32-7] - BSA and Azide free ab213216

KO VALIDATED Recombinant RabMAb

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Overview

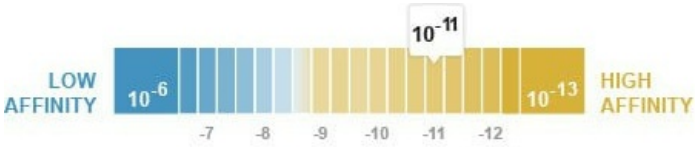
Product name	Anti-Cdk4 antibody [EPR4513-32-7] - BSA and Azide free
Description	Rabbit monoclonal [EPR4513-32-7] to Cdk4 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, ICC/IF, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1 and HeLa cell lysates, MCF7 membrane extract lysate (**ab29539**). Flow Cyt (intra): MCF-7 IHC-P: Human cervix carcinoma and human urothelial carcinoma. ICC/IF: MCF-7 and HAP1.
General notes	ab213216 is the carrier-free version of **ab108357**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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. Do Not Freeze.
Dissociation constant (K_D)	$K_D = 1.86 \times 10^{-11}$ M



Learn more about K_D

Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR4513-32-7
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab213216 in the following tested applications.

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

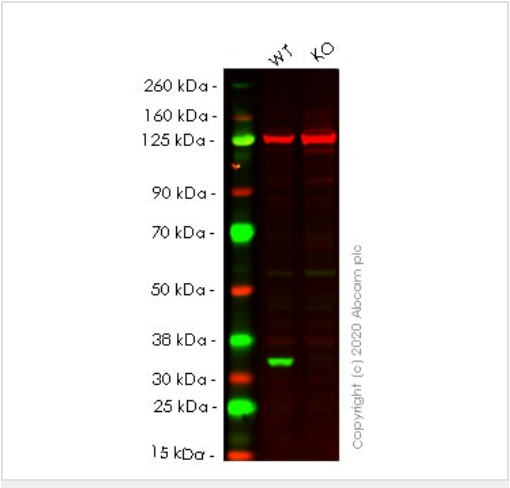
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Detects a band of approximately 34 kDa (predicted molecular weight: 34 kDa).
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.

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] - BSA and Azide free (ab213216)

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.

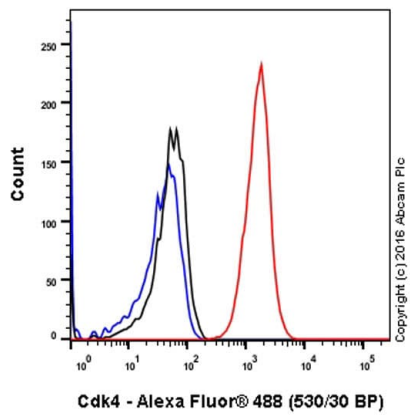
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (**ab108357**).

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.



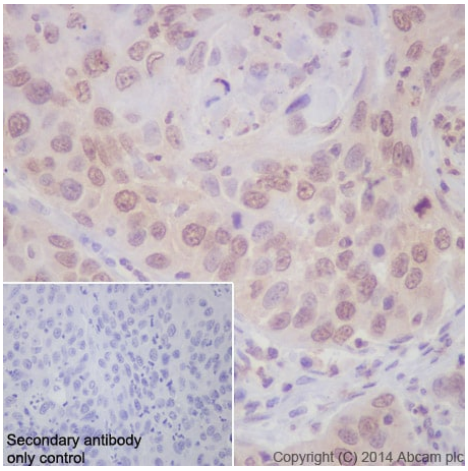
Flow Cytometry (Intracellular) - Anti-Cdk4 antibody [EPR4513-32-7] - BSA and Azide free (ab213216)

ab108357 staining CDK4 in the human cell line MCF-7 (human breast carcinoma) 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)

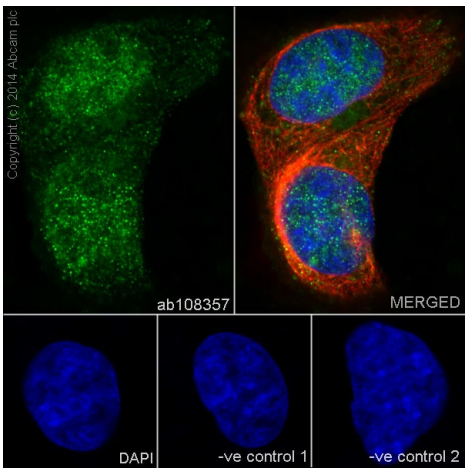
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108357**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cdk4 antibody [EPR4513-32-7] - BSA and Azide free (ab213216)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108357**).



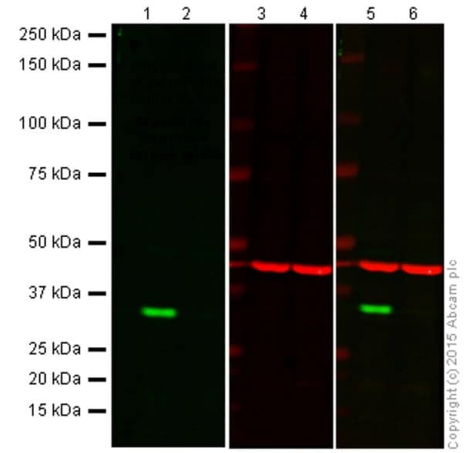
Immunocytochemistry/ Immunofluorescence - Anti-Cdk4 antibody [EPR4513-32-7] - BSA and Azide free (ab213216)

Immunocytochemical analysis of MCF7 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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108357**).



Western blot - Anti-Cdk4 antibody [EPR4513-32-7] - BSA and Azide free (ab213216)

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

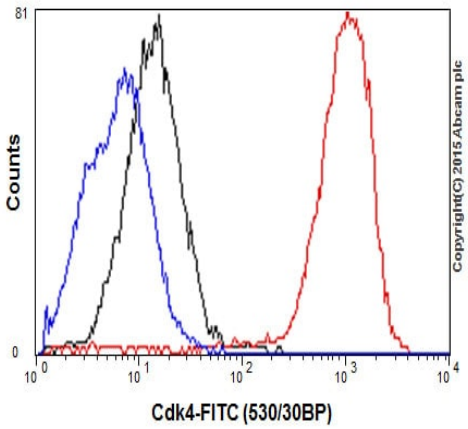
Lysates/proteins at 20 µg per lane.

Predicted band size: 34 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab108357**).

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

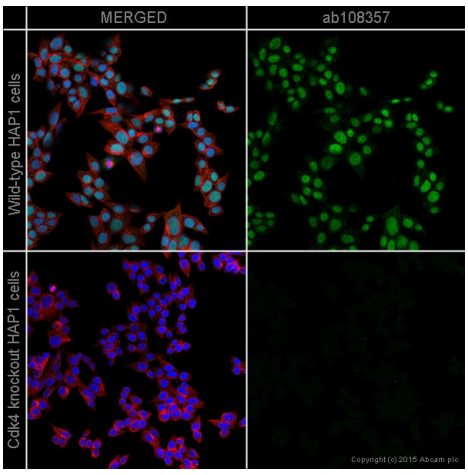
ab108357 was shown to specifically react with CDK4 in wild-type HAP1 cells. No band was observed when CDK4 knockout samples were examined. Wild-type and CDK4 knockout samples were subjected to SDS-PAGE. **ab108357** and **ab8226** (loading control to beta actin) were both diluted at 1/1000 and incubated 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.



Flow Cytometry (Intracellular) - Anti-Cdk4 antibody [EPR4513-32-7] - BSA and Azide free (ab213216)

Intracellular Flow Cytometry analysis of MCF7 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.

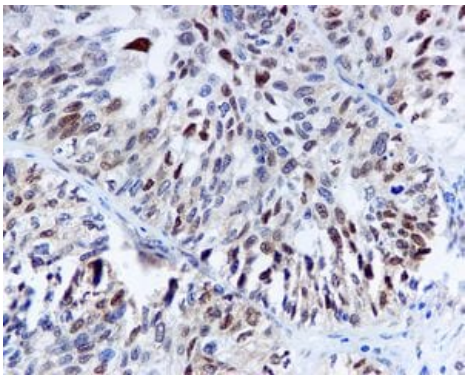
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108357**).



Immunocytochemistry/ Immunofluorescence - Anti-Cdk4 antibody [EPR4513-32-7] - BSA and Azide free (ab213216)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108357**).

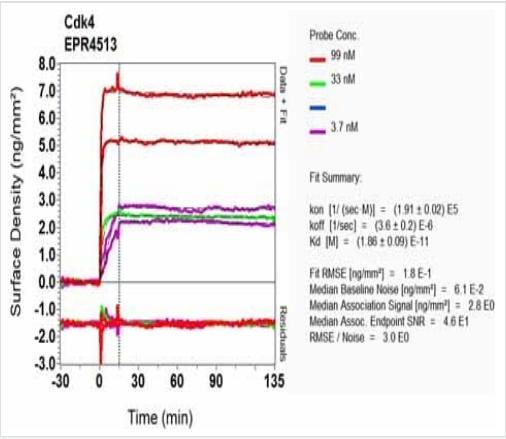


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cdk4 antibody [EPR4513-32-7] - BSA and Azide free (ab213216)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108357**).



OI-RD Scanning - Anti-Cdk4 antibody [EPR4513-32-7] - BSA and Azide free (ab213216)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108357**).

Anti-Cdk4 antibody [EPR4513-32-7] - BSA and Azide free (ab213216)

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