abcam

ab213216

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

Recombinant | RabMAb | KO Validated

## Key facts

| | |
|---|---|
| **Isotype** | IgG |
| **Host species** | Rabbit |
| **Storage buffer** | pH: 7.2 - 7.4 Constituents: PBS |
| **Form** | Liquid |
| **Clonality** | Monoclonal |
| **Immunogen** | The exact immunogen used to generate this antibody is proprietary information. |
| **Carrier free** | Yes |
| **Clone number** | EPR4513-32-7 |
| **Purification technique** | Affinity purification Protein A |
| **Dissociation constant** | 1.86 x $10^{-11}$ M |
| **Concentration** | 0.989 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |

## Reactivity data

## IHC-P

**Tested**

| | |
|---|---|
| **Species** | Human |

| | |
|---|---|
| **Dilution info** | - |
| **Notes** | - |

## WB

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | - |

## ICC/IF

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | - |

## Flow Cyt (Intra)

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. |

# Target data

See full target information CDK4

**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.

## Storage

| | |
|---|---|
| **Shipped at conditions** | Blue Ice |
| **Appropriate short-term storage conditions** | +4°C |
| **Appropriate long-term storage conditions** | +4°C |
| **Storage information** | Do Not Freeze |

## Notes

ab213216 is the carrier-free version of ab108357.

**Species reactivity**

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

**Patented technology**

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

**What are the advantages of a recombinant monoclonal antibody?**

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 batch production

For more information, read more on recombinant antibodies.

**Conjugation ready**

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

**Compatibility**

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.

## Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

| | |
|---|---|
| **Activity summary** | Cdk4 also known as Cyclin-dependent kinase 4 is a serine/threonine kinase involved in cell cycle regulation. Cdk4 has a molecular weight of approximately 34 kDa. This protein mainly locates in the nucleus of the cell and its expression occurs in diverse tissues. Cdk4 partners with D-type cyclins to exert its function which is essential for the transition from the G1 phase to the S phase of the cell cycle. Researchers often study Cdk4 using techniques like immunohistochemistry (IHC) to determine its expression and localization in different tissues. |
| **Biological function summary** | Cdk4 plays a fundamental role in cell cycle regulation by forming a complex with D-type cyclins. Together they phosphorylate the retinoblastoma protein (Rb) resulting in the release of E2F transcription factors which promote the expression of genes necessary for DNA replication. The Cdk4/cyclin D complex regulates the cell's commitment to enter S phase and continue cell division. This regulation is key for normal cell proliferation and tissue homeostasis. Cdk4 activity is strictly controlled by INK4 family members acting as inhibitors and adding an extra regulation layer. |
| **Pathways** | Cdk4 is an integral part of important signaling pathways like the cell cycle and PI3K/AKT pathways. In the cell cycle pathway Cdk4 relays signals downstream that drive the transition from the G1 to S phase through its interaction with cyclin D and Rb. In the PI3K/AKT pathway signaling can influence cyclin D expression indirectly affecting Cdk4 activity. Proteins such as Cdk6 closely relate to Cdk4 and often compensate or partner with Cdk4 in these pathways to maintain proper cell cycle progression. |
| **Associated diseases and disorders** | Abnormalities in Cdk4 function or regulation relate to cancer and certain metabolic syndromes. Hyperactivity of Cdk4 due to overexpression or mutation frequently occurs in several cancers including melanoma and breast cancer. In these malignancies Cdk4 aberrantly drives excessive cell proliferation. Mutations or alterations in proteins such as p16 an inhibitor of Cdk4 are often found in these cancers highlighting the critical role of Cdk4 in disease progression. Understanding the role of Cdk4 allows researchers to develop targeted therapies such as Cdk4/6 inhibitors offering new treatment options. |

## Product promise

**Tested**

We have tested this species and application combination and it works. It is covered by our product promise.

**Expected**

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

**Predicted**

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

**Not recommended**

We do not recommend this combination. It is not covered by our product promise.

---

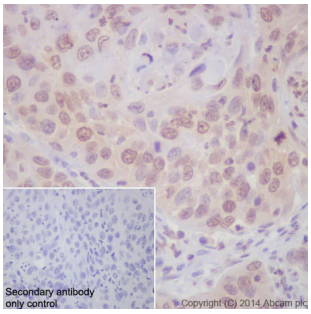
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

---

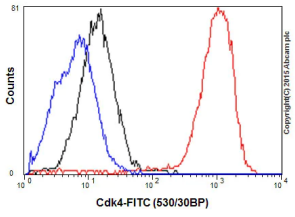
## 9 product images




### 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).

---

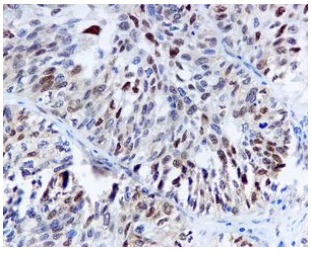



### Flow Cytometry (Intracellular) - Anti-Cdk4 antibody [EPR4513-32-7] - BSA and Azide free (ab213216)

Cdk4 Flow Cytometry (Intracellular) staining using rabbit Anti-Cdk4 antibody

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).

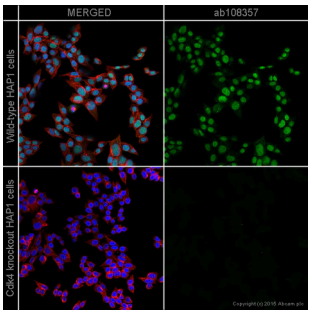
---



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

Cdk4 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Cdk4 antibody

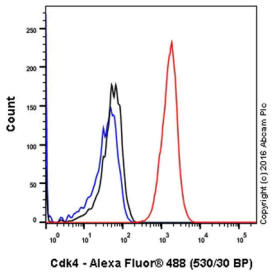
Immunohistochemical analysis of formalin/PFA-fixed paraffin-embedded human urothelial carcinoma tissue labelling Cdk4 with unpurified ab108357 at a dilution of 1/100.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab108357).




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

Cdk4 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Cdk4 antibody

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).



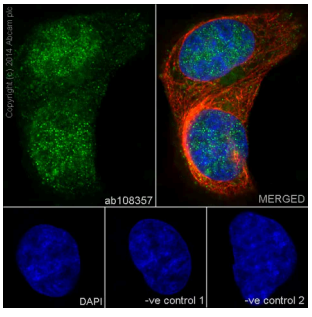

## Flow Cytometry (Intracellular) - Anti-Cdk4 antibody [EPR4513-32-7] - BSA and Azide free (ab213216)

Cdk4 Flow Cytometry (Intracellular) staining using rabbit Anti-Cdk4 antibody

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).



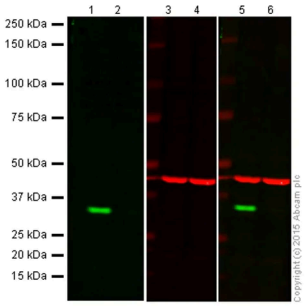

## Immunocytochemistry/ Immunofluorescence - Anti-Cdk4 antibody [EPR4513-32-7] - BSA and Azide free (ab213216)

Cdk4 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Cdk4 antibody

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)

Cdk4 Western blot staining using rabbit Anti-Cdk4 antibody

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.

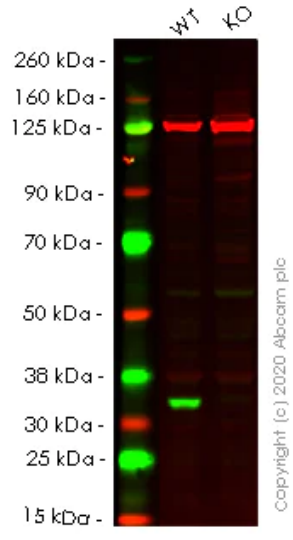
Lanes 1 - 4:
Western blot - Anti-Cdk4 antibody [EPR4513-32-7] - BSA and Azide free (ab213216) at 1/1000 dilution

Lanes 5 - 6:
Western blot - Anti-Cdk4 antibody [EPR4513-32-7] - BSA and Azide free (ab213216)

Lanes 1, 3 and 5:
Wild-type HAP1 cell lysate at 20 µg

Lanes 2, 4 and 6:
CDK4 knockout HAP1 cell lysate at 20 µg

Predicted band size: 34 kDa




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

Cdk4 Western blot staining using rabbit Anti-Cdk4 antibody

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.

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

Lane 1:
Wild-type HeLa cell lysate at 20 µg

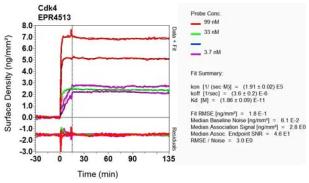
Lane 2:
CDK4 knockout HeLa cell lysate at 20 µg

Lane 2:
Western blot - Human CDK4 knockout HeLa cell line (ab255378)

Performed under reducing conditions.

Predicted band size: 34 kDa, 55 kDa

Observed band size: 34 kDa, 55 kDa




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

We have systematically measured KD (the equilibrium dissociation constant between the antibody and its antigen), of more than 840 recombinant antibodies to assess not only their individual KD values but also to see the average affinity of antibody. Based on the comparison with published literature values for mouse monoclonal antibodies, Recombinant antibodies appear to be on average 1-2 order of magnitude higher affinity.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.