abcam

ab236042

Anti-Cdk4 antibody [EPR2513Y] - 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	EPR2513Y
Purification technique	Affinity purification Protein A
Concentration	1.066 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	-

Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-

Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

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

ab236042 is the carrier-free version of ab68266.

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

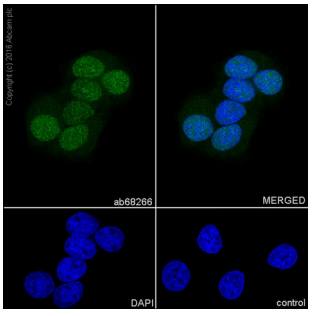
We do not recommend this combination. It is not covered by our product promise.

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.

6 product images

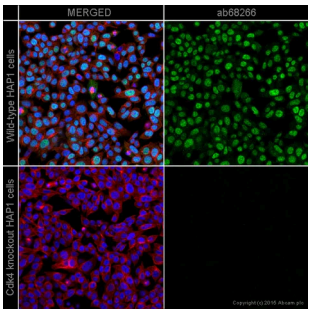


Immunocytochemistry/ Immunofluorescence - Anti-Cdk4 antibody [EPR2513Y] - BSA and Azide free (ab236042)

Cdk4 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Cdk4 antibody

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

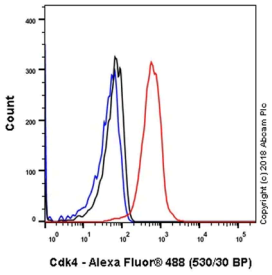
used as the secondary antibody only control.This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab68266).



Immunocytochemistry/ Immunofluorescence - Anti-Cdk4 antibody [EPR2513Y] - BSA and Azide free (ab236042)

Cdk4 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Cdk4 antibody

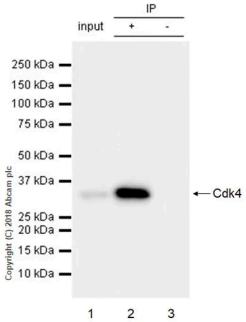
Unpurified ab68266 staining Cdk4 in wild-type HAP1 cells (top panel) and Cdk4 knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab68266).



Flow Cytometry (Intracellular) - Anti-Cdk4 antibody [EPR2513Y] - BSA and Azide free (ab236042)

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

Intracellular 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). This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab68266).

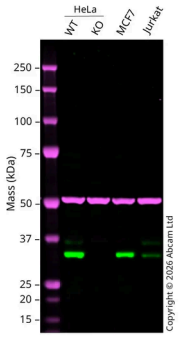


Immunoprecipitation - Anti-Cdk4 antibody [EPR2513Y] - BSA and Azide free (ab236042)

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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab68266).

All lanes:
Immunoprecipitation - Anti-Cdk4 antibody [EPR2513Y] (ab68266)

Predicted band size: 34 kDa



Western blot - Anti-Cdk4 antibody [EPR2513Y] - BSA and Azide free (ab236042)

Cdk4 Western blot staining using rabbit Anti-Cdk4 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab68266).

Western blot: Anti-Cdk4 antibody [EPR2513Y] ab68266 staining at 1/2000 dilution, shown in green; Mouse anti alpha Tubulin ab7291 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 34 kDa in Wild-type HeLa cell lysates with no signal observed at this size in CDK4 knockout HeLa cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5% Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-Cdk4 antibody [EPR2513Y] (ab68266) at 1/2000 dilution

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

Lane 2:
Western blot - Human CDK4 knockout HeLa cell line (ab255378) at 20 µg

Lane 3:
MCF7 cell lysates at 20 µg

Lane 4:
Jurkat cell lysates at 20 µg

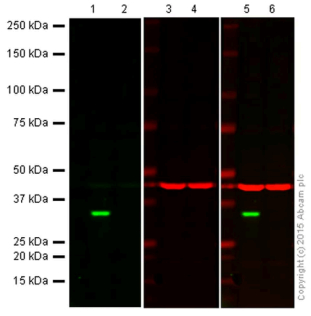
Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 34 kDa

Observed band size: 34 kDa



Western blot - Anti-Cdk4 antibody [EPR2513Y] - BSA and Azide free (ab236042)

Cdk4 Western blot staining using rabbit Anti-Cdk4 antibody

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab68266).
All lanes:
Western blot - Anti-Cdk4 antibody [EPR2513Y] - BSA and Azide free (ab236042)

Predicted band size: 34 kDa

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