abcam

ab222395

Anti-Cdk6 antibody [EPR4515] - 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	EPR4515
Purification technique	Affinity purification Protein A
Specificity	Based on the immunogen sequence, we do not expect the antibody to cross-react with other CDK family members. No cross-reactivity testing has been performed.
Concentration	1.006 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human

Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

IP

Not recommended

Species	Human
Dilution info	-
Notes	-

WB

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 CDK6

Function	Serine/threonine-protein kinase involved in the control of the cell cycle and differentiation; promotes G1/S transition. Phosphorylates pRB/RB1 and NPM1. Interacts with D-type G1 cyclins during interphase at G1 to form a pRB/RB1 kinase and controls the entrance into the cell cycle. Involved in initiation and maintenance of cell cycle exit during cell differentiation; prevents cell proliferation and negatively regulates cell differentiation, but is required for the proliferation of specific cell types (e.g. erythroid and hematopoietic cells). Essential for cell proliferation within the dentate gyrus of the hippocampus and the subventricular zone of the lateral ventricles. Required during thymocyte development. Promotes the production of newborn neurons, probably by modulating G1 length. Promotes, at least in astrocytes, changes in patterns of gene expression, changes in the actin cytoskeleton including loss of stress fibers, and enhanced motility during cell differentiation. Prevents myeloid differentiation by interfering with RUNX1 and reducing its transcription transactivation activity, but promotes proliferation of normal myeloid progenitors. Delays senescence. Promotes the proliferation of beta-cells in pancreatic islets of Langerhans. May play a role in the centrosome organization during the cell cycle phases (PubMed:23918663).

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

ab222395 is the carrier-free version of ab124821.

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.

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	Cdk6 also known as Cyclin-dependent kinase 6 is a protein kinase involved in the regulation of the cell cycle. It has a molecular weight of approximately 40 kDa. Cdk6 is expressed in various tissues including lymphoid tissues and the brain. It plays a mechanical role by phosphorylating target proteins which impacts cell cycle progression particularly during the transition from G1 phase to S phase. Cdk6 can work in conjunction with D-type cyclins to form a complex that is critical for its activity.
Biological function summary	Cdk6 functions as a regulator of the cell cycle. It participates in a complex with cyclin D to control the G1 phase progression. This activity is essential for the regulation of cell division and proliferation. The association with cyclin D allows Cdk6 to phosphorylate the retinoblastoma protein (Rb) leading to the release of E2F transcription factors that promote the expression of genes necessary for DNA synthesis.
Pathways	Cdk6 is an important component of the cell cycle control pathway. It interacts primarily with the retinoblastoma protein and Cyclin D1 in this context. The cell cycle pathway is critical for controlled cell proliferation and its dysregulation can lead to diseases like cancer. Another involved pathway is the PI3K/AKT pathway which can activate Cdk6 activity through upstream signaling events implicating Cdk6 in cellular responses to growth signals.
Associated diseases and disorders	Cdk6 has a significant role in cancer development due to its involvement in uncontrolled cell proliferation. Its overexpression or dysregulation is often linked to cancers such as leukemia and glioblastoma. In cancer the interaction with proteins like cyclin D1 can lead to unchecked progression through the cell cycle contributing to oncogenesis. Additionally Cdk6 inhibitors are being explored as therapeutic agents in cancer treatment due to their potential to restore control over cell cycle progression.

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.

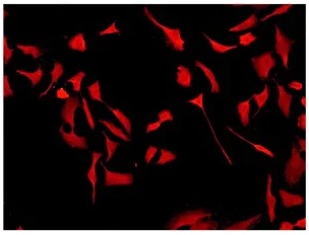
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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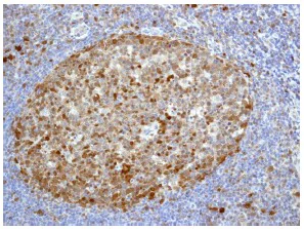
9 product images



Immunocytochemistry/ Immunofluorescence - Anti-Cdk6 antibody [EPR4515] - BSA and Azide free (ab222395)

Cdk6 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Cdk6 antibody

ICC/IF image of ab124821 at 1/100 dilution, staining Cdk6 in HeLa cells.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab124821).

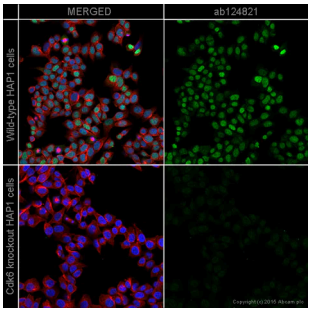


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cdk6 antibody [EPR4515] - BSA and Azide free (ab222395)

Cdk6 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Cdk6 antibody

ab124821 at 1/100 dilution, staining Cdk6 in formalin-fixed paraffin-embedded human tonsil tissue by immunohistochemistry.
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

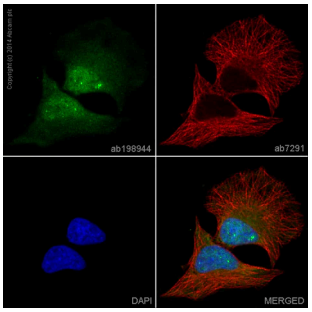
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab124821).



Immunocytochemistry/ Immunofluorescence - Anti-Cdk6 antibody [EPR4515] - BSA and Azide free (ab222395)

Cdk6 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Cdk6 antibody

ab124821 staining Cdk6 in wild-type HAP1 cells (top panel) and Cdk6 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 ab124821 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 (ab124821).

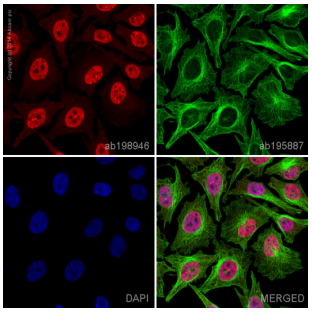


Immunocytochemistry/ Immunofluorescence - Anti-Cdk6 antibody [EPR4515] - BSA and Azide free (ab222395)

Cdk6 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Cdk6 antibody

Clone EPR4515 (ab222395) has been successfully conjugated by Abcam. This image was generated using Anti-Cdk6 antibody [EPR4515] (Alexa Fluor® 488). Please refer to ab198944 for protocol details.
ab198944 staining Cdk6 in HeLa cells. The cells were fixed with 100% methanol (5min), 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 overnight at +4°C with ab198944 at 1/100 dilution (shown in green) and ab7291 (Mouse monoclonal [DM1A] to alpha Tubulin) at 1µg/ml. This was followed by an incubation at room temperature for 1h with ab150120, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed, at 1µg/ml (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



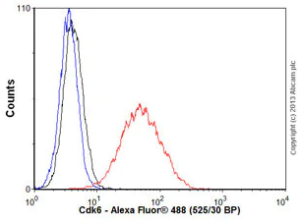
Immunocytochemistry/ Immunofluorescence - Anti-Cdk6 antibody [EPR4515] - BSA and Azide free (ab222395)

Cdk6 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Cdk6 antibody

Clone EPR4515 (ab222395) has been successfully conjugated by Abcam. This image was generated using Anti-Cdk6 antibody [EPR4515] (Alexa Fluor® 647). Please refer to ab198946 for protocol details.
ab198946 staining Cdk6 in HeLa cells. The cells were fixed with 4% formaldehyde (10 min), 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 overnight at +4°C with ab198946 at 1/100 dilution (shown in red) and ab195887, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

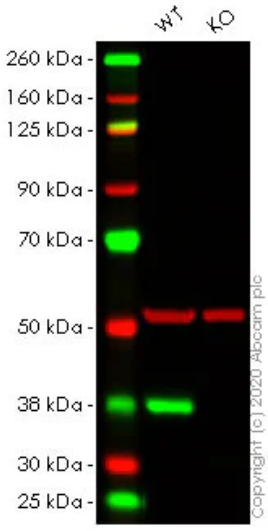
This product also gave a positive signal under the same testing conditions in HeLa cells fixed with 100% methanol (5min).



Flow Cytometry (Intracellular) - Anti-Cdk6 antibody [EPR4515] - BSA and Azide free (ab222395)

Cdk6 Flow Cytometry (Intracellular) staining using rabbit Anti-Cdk6 antibody

Overlay histogram showing HeLa cells stained with ab124821 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab124821, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab124821).



Western blot - Anti-Cdk6 antibody [EPR4515] - BSA and Azide free (ab222395)

This data was developed using the same antibody clone in a different buffer formulation (ab124821).
Lanes 1- 2: Merged signal (red and green). Green - ab124821 observed at 37 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) observed at 50 kDa.

ab124821 was shown to react with Cdk6 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab266059 (knockout cell lysate ab257088) was used. Wild-type HeLa and CDK6 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. ab124821 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) 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-Cdk6 antibody [EPR4515] (ab124821) at 1/1000 dilution

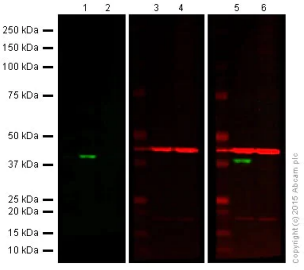
Lane 1:
Wild-type HeLa cell lysate at 20 µg

Lane 2:
CDK6 knockout HeLa cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 37 kDa

Observed band size: 37 kDa



Western blot - Anti-Cdk6 antibody [EPR4515] - BSA and Azide free (ab222395)

Cdk6 Western blot staining using rabbit Anti-Cdk6 antibody

Lanes 1 and 2: Green signal from target - ab124821 observed at 37 kDa
Lanes 3 and 4: Red signal from loading control - ab8226 observed at 42 kDa

Lanes 5 and 6: Merged (red and green) signal
ab124821 was shown to specifically react with CDK6 when CDK6 knockout samples were used. Wild-type and CDK6 knockout samples were subjected to SDS-PAGE. ab124821 and ab8226 (loading control to beta actin) were diluted at 1/10 000 and 1/1000 respectively 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 h at room temperature before imaging.
Lanes 1 - 2:
Western blot - Anti-Cdk6 antibody [EPR4515] - BSA and Azide free (ab222395) at 1/10000 dilution

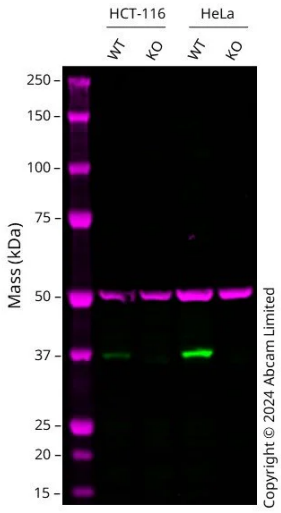
Lanes 3 - 4:
Western blot - Anti-Cdk6 antibody [EPR4515] - BSA and Azide free (ab222395) at 1/1000 dilution

Lanes 5 - 6:
Western blot - Anti-Cdk6 antibody [EPR4515] - BSA and Azide free (ab222395)

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

Lanes 2, 4 and 6:
CDK6 knockout HAP1 cell lysate at 20 µg

Predicted band size: 37 kDa



Western blot - Anti-Cdk6 antibody [EPR4515] - BSA and Azide free (ab222395)

Cdk6 Western blot staining using rabbit Anti-Cdk6 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab124821).

Western blot: Anti-CDK6 antibody [EPR4515] (ab124821) staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab124821 was shown to bind specifically to CDK6. A band was observed at 37 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in CDK6 knockout cell line. To generate this image, wild-type and CDK6 knockout HCT 116 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % 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 IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

All lanes:
Western blot - Anti-Cdk6 antibody [EPR4515] (ab124821) at 1/1000 dilution

Lane 1:
Wild-type HCT 116 cell lysate at 20 µg

Lane 2:
CDK6 knockout HCT 116 cell lysate at 20 µg

Lane 3:
Wild-type HeLa ab255929 cell lysate at 20 µg

Lane 4:
CDK6 knockout HeLa ab260923 cell lysate at 20 µg

Secondary

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

Performed under reducing conditions.

Predicted band size: 37 kDa

Observed band size: 37 kDa

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