abcam

Product datasheet

Anti-Cdk6 antibody [EPR4515] - BSA and Azide free ab222395

KO 評価済 リコンビナント RabMAb

★★★★★ 2 Abreviews 6 References 画像数 9

製品の概要

製品名	Anti-Cdk6 antibody [EPR4515] - BSA and Azide free
製品の詳細	Rabbit monoclonal [EPR4515] to Cdk6 - BSA and Azide free
由来種	Rabbit
特異性	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.
アプリケーション	**適用あり:** Flow Cyt (Intra), ICC/IF, WB, IHC-P **適用なし:** IP
種交差性	**交差種:** Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: Jurkat, K562, HeLa, HAP1, and 293T cell lysates. IHC-P:Human tonsil tissue. ICC/IF: HeLa and HAP1 cells.
特記事項	ab222395 is the carrier-free version of **ab124821**. 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. 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.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C. Do Not Freeze.
バッファー	pH: 7.2 Constituent: PBS
キャリア・フリー	はい
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR4515
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab222395の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

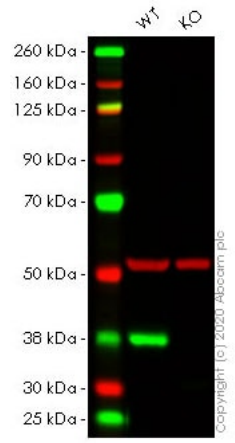
アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★★ (1)	Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 37 kDa (predicted molecular weight: 37 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

追加情報 Is unsuitable for IP.

ターゲット情報

機能	Probably involved in the control of the cell cycle. Interacts with D-type G1 cyclins.
配列類似性	Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. CDC2/CDKX subfamily. Contains 1 protein kinase domain.

画像



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

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

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : CDK6 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

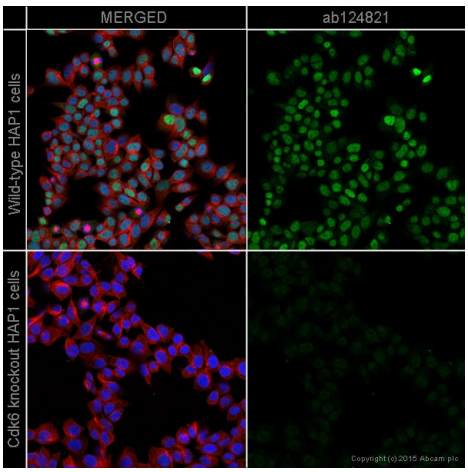
Performed under reducing conditions.

Predicted band size: 37 kDa
Observed band size: 37 kDa

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.

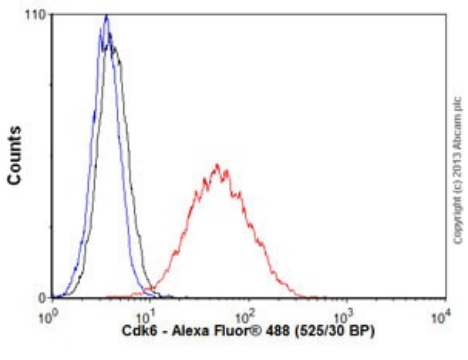
ab124821 was shown to react with Cdk6 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab266059** (knockout cell lysate **ab257088**) was used. Wild-type HeLa and CDK6 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour 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.



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

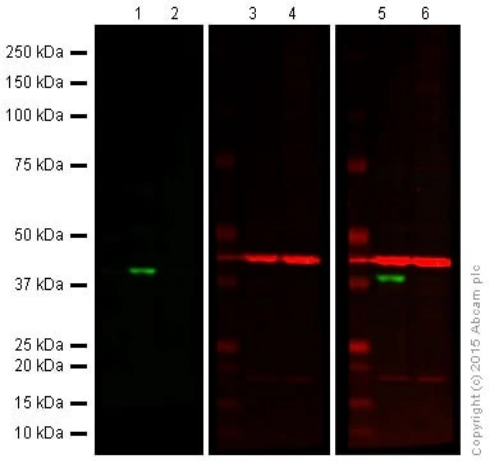
ab124821 staining Cdk6 in wild-type HAP1 cells (top panel) and Cdk6 knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells 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**).



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

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)

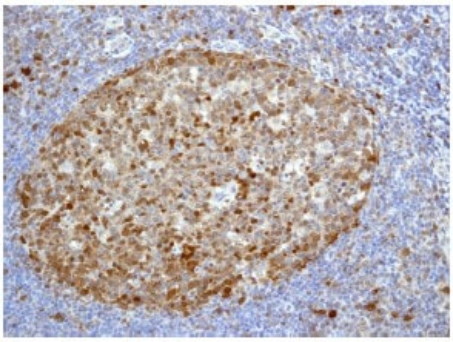
Lanes 1-2 : Anti-Cdk6 antibody [EPR4515] (**ab124821**) at 1/10000 dilution
Lanes 3-4 : Anti-beta Actin antibody [mAbcam 8226] - Loading Control (**ab8226**) at 1/1000 dilution

Lanes 1 & 3 & 5 : Wild-type HAP1 cell lysate
Lanes 2 & 4 & 6 : CDK6 knockout HAP1 cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 37 kDa

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.

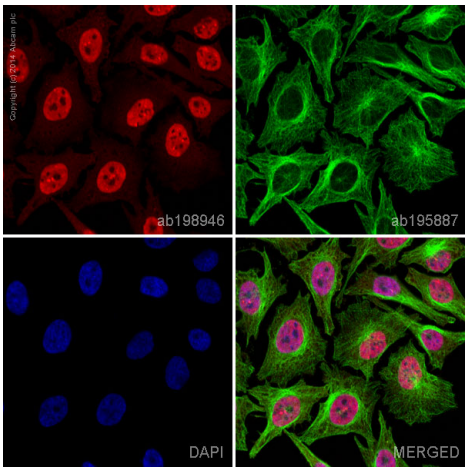


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cdk6 antibody [EPR4515] - BSA and Azide free (ab222395)

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**).



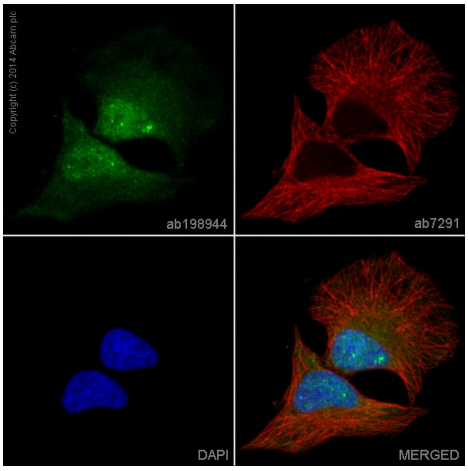
Immunocytochemistry/ Immunofluorescence - Anti-Cdk6 antibody [EPR4515] - BSA and Azide free (ab222395)

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).

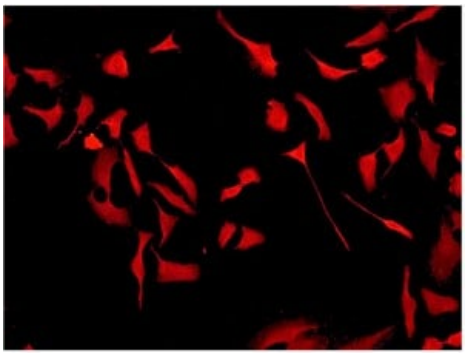


Immunocytochemistry/ Immunofluorescence - Anti-Cdk6 antibody [EPR4515] - BSA and Azide free (ab222395)

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).

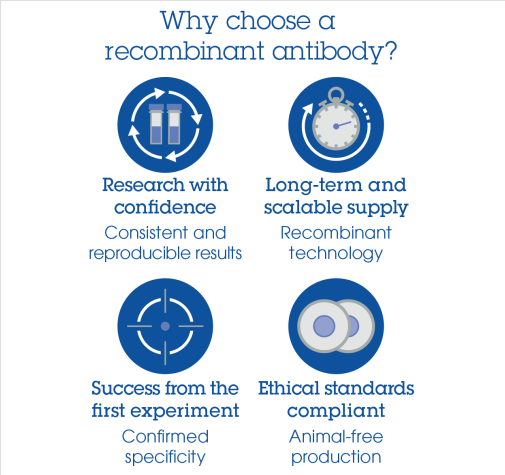
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



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

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**).



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

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