abcam

Product datasheet

Anti-Cdk6 antibody [EPR4515] ab124821





重组 RabMAb

★★★★★ 2 Abreviews 91 References 8 图像

概述

产品名称 Anti-Cdk6抗体[EPR4515]

描述 兔单克隆抗体[EPR4515] to Cdk6

宿主 Rabbit

特异性 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.

经测试应用 适用于: ICC/IF, WB, IHC-P, Flow Cyt (Intra)

不适用于: №

种属反应性 与反应: Human

免疫原 Synthetic peptide. within Human Cdk6 aa 300 to the C-terminus (C terminal). The exact sequence

is proprietary.

Database link: Q00534

阳性对照 WB: Jurkat, K562, HAP1, HeLa and 293T cell lysates. IHC-P: Human tonsil tissue. ICC/IF: HeLa

and wild-type HAP1 cells.

常规说明 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 $\mathsf{RabMAb}^{\texttt{®}}$ 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.

性能

形式 Liquid

存放说明 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Stable for 12 months at -20°C.

1

存储溶液 pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA

纯**度** Protein A purified

同种型 IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab124821于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ICC/IF		1/500.
WB	*****(1)	1/50000 - 1/200000. Detects a band of approximately 37 kDa (predicted molecular weight: 37 kDa).
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/100 - 1/1000. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

应用说明 Is unsuitable for IP.

靶标

功能 Probably involved in the control of the cell cycle. Interacts with D-type G1 cyclins.

序列相似性 Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. CDC2/CDKX

subfamily.

Contains 1 protein kinase domain.

图片



Western blot - Anti-Cdk6 antibody [EPR4515] (ab124821)

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

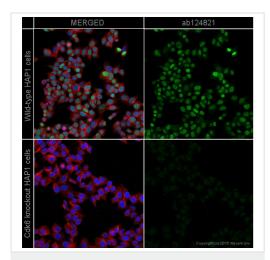
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

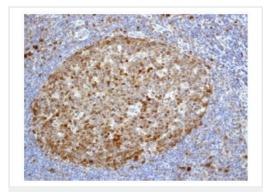
Lanes 1-2: Merged signal (red and green). Green - ab124821 observed at 37 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) observed at 50 kDa.

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 lgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



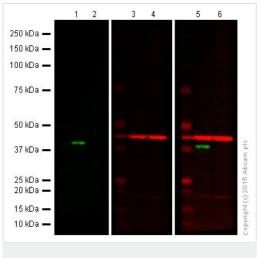
Immunocytochemistry/ Immunofluorescence - Anti-Cdk6 antibody [EPR4515] (ab124821)

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 μ g/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cdk6 antibody
[EPR4515] (ab124821)

ab124821 at 1/100 dilution, staining Cdk6 in Formalin fixed Paraffin-embedded Human tonsil tissue by Immunohistochemistry. Heat mediated antigen retrieval was performed with 0.01M citrate buffer, pH 6.0.



Western blot - Anti-Cdk6 antibody [EPR4515] (ab124821)

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

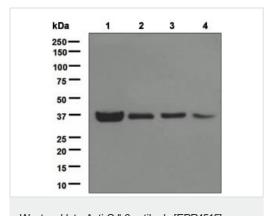
Lysates/proteins at 20 µg per lane.

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 - <u>ab8226</u> 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.



Western blot - Anti-Cdk6 antibody [EPR4515] (ab124821)

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

Lane 1: Jurkat cell lysate

Lane 2: K562 cell lysate

Lane 3: HeLa cell lysate

Lane 4: 293T cell lysate

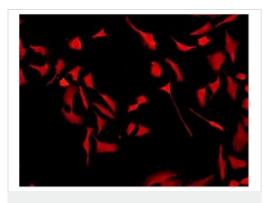
Lysates/proteins at 10 µg per lane.

Secondary

All lanes: Goat-anti-rabbit HRP at 1/2000 dilution

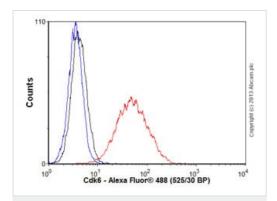
Predicted band size: 37 kDa

Observed band size: 37 kDa



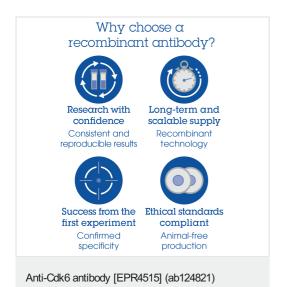
Immunocytochemistry/ Immunofluorescence - Anti-Cdk6 antibody [EPR4515] (ab124821)

ICC/IF image of ab124821 at 1/100 dilution, staining Cdk6 in HeLa cells.



Flow Cytometry (Intracellular) - Anti-Cdk6 antibody [EPR4515] (ab124821)

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 lgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (0.1 μ g/1x106 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.



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