abcam

Product datasheet

Anti-Cdk4 antibody [EPR4513-54-3] ab108355



★★★★★ 1 Abreviews 4 References 画像数 6

製品の概要

製品名 Anti-Cdk4 antibody [EPR4513-54-3]

製品の詳細 Rabbit monoclonal [EPR4513-54-3] to Cdk4

由来種 Rabbit

アプリケーション 適用あり: WB, ICC/IF

適用なし: IHC-P

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: HeLa, MCF7 and K562 cell lysates. ICC/IF: Wild-type HAP1 and HeLa cells.

特記事項 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 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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Stable for 12 months at -20°C.

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS

精製度 Protein A purified

ポリモノ モノクローナル

アイソタイプ

lgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab108355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
WB	****(1)	1/1000 - 1/10000. Detects a band of approximately 34 kDa (predicted molecular weight: 34 kDa).
ICC/IF		1/400. This product gave a positive signal in HAP1 cells fixed with 4% formaldehyde (10 min) and 100% methanol (5 min)

追加情報

Is unsuitable for IHC-P.

ターゲット情報

機能

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.

関連疾患

Defects in CDK4 are a cause of susceptibility to cutaneous malignant melanoma type 3 (CMM3) [MIM:609048]. Malignant melanoma is a malignant neoplasm of melanocytes, arising de novo or from a pre-existing benign nevus, which occurs most often in the skin but also may involve other sites.

配列類似性

Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. CDC2/CDKX subfamily.

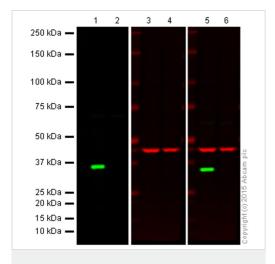
Contains 1 protein kinase domain.

翻訳後修飾

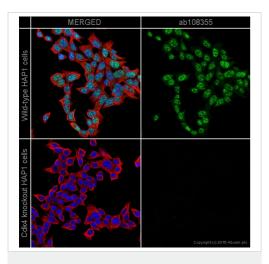
Phosphorylation at Thr-172 is required for enzymatic activity. Phosphorylated, in vitro, at this site by CCNH-CDK7, but, in vivo, appears to be phosphorylated by a proline-directed kinase. In the cyclin D-CDK4-CDKN1B complex, this phosphorylation and consequent CDK4 enzyme activity, is dependent on the tyrosine phosphorylation state of CDKN1B. Thus, in proliferating cells, CDK4 within the complex is phosphorylated on Thr-172 in the T-loop. In resting cells, phosphorylation on Thr-172 is prevented by the non-tyrosine-phosphorylated form of CDKN1B.

細胞内局在

Cytoplasm. Nucleus. Membrane. Cytoplasmic when non-complexed. Forms a cyclin D-CDK4 complex in the cytoplasm as cells progress through G(1) phase. The complex accumulates on the nuclear membrane and enters the nucleus on transition from G(1) to S phase. Also present in nucleoli and heterochromatin lumps. Colocalizes with RB1 after release into the nucleus.



Western blot - Anti-Cdk4 antibody [EPR4513-54-3] (ab108355)



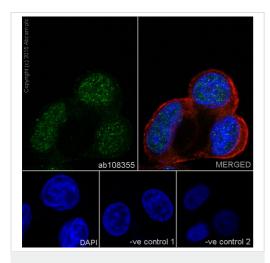
Immunocytochemistry/ Immunofluorescence - Anti-Cdk4 antibody [EPR4513-54-3] (ab108355)

Lanes 1, 3 and 5: Wild-type HAP1 cell lysate (20 μ g) Lanes 2, 4 and 6: CDK4 knockout HAP1 cell lysate (20 μ g) Lanes 1 and 2: Green signal from target - ab108355 observed at 34 kDa

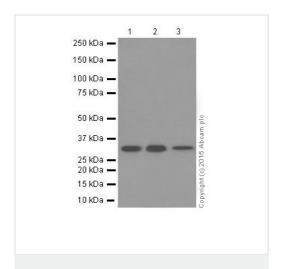
Lanes 3 and 4: Red signal from loading control - <u>ab8226</u> observed at 42 kDa

Lanes 5 and 6: Merged (red and green) signal ab108355 was shown to specifically react with CDK4 when CDK4 knockout samples were used. Wild-type and CDK4 knockout samples were subjected to SDS-PAGE. ab108355 and <u>ab8226</u> (loading control to beta actin) were both diluted at 1/1000 and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging.

ab108355 staining Cdk4 in wild-type HAP1 cells (top panel) and Cdk4 knockout HAP1 cells (bottom panel). 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 with ab108355 at 1/400 dilution and ab7291 at 1µg/ml concentration overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit lgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green) and a goat secondary antibody to Mouse lgG (Alexa Fluor® 594) (ab150120) (shown in pseudo colour red). Nuclear DNA was labelled in blue with DAPI.



Immunocytochemistry/ Immunofluorescence - Anti-Cdk4 antibody [EPR4513-54-3] (ab108355)



Western blot - Anti-Cdk4 antibody [EPR4513-54-3] (ab108355)

Immunofluorescence staining of MCF7 cells with purified ab108355 at a working dilution of 1/100, counter-stained with DAPI. The secondary antibody was Alexa Fluor[®] 488 goat anti-rabbit (ab150077), used at a dilution of 1/1000. ab7291, a mouse antitubulin antibody (1/1000), was used to stain tubulin along with ab150120 (Alexa Fluor[®] 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab108355 was used at a dilution of 1/500 followed by an Alexa Fluor[®] 594 goat anti-mouse antibody (ab150120) at a dilution of 1/500. For negative control 2, ab7291 (mouse antitubulin) was used at a dilution of 1/500 followed by an Alexa Fluor[®] 488 goat anti-rabbit antibody (ab150077) at a dilution of 1/400.

All lanes : Anti-Cdk4 antibody [EPR4513-54-3] (ab108355) at 1/5000 dilution (purified)

Lane 1 : HeLa cell lysate
Lane 2 : K562 cell lysate
Lane 3 : Ramos cell lysate

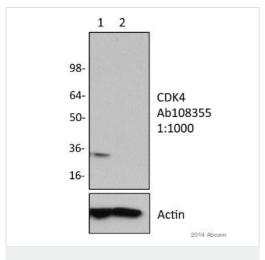
Lysates/proteins at 10 µg per lane.

Secondary

All lanes: HRP goat anti-rabbit lgG (H+L) at 1/50000 dilution

Predicted band size: 34 kDa Observed band size: 34 kDa

Blocking buffer: 5% NFDM/TBST Dilution buffer: 5% NFDM/TBST



Western blot - Anti-Cdk4 antibody [EPR4513-54-3] (ab108355)

This image is courtesy of an Abreview submitted by Sonia Rocha

All lanes : Anti-Cdk4 antibody [EPR4513-54-3] (ab108355) at 1/1000 dilution (unpurified)

Lane 1: Human osteosarcoma cells - control, non-targeting siRNA

Lane 2: Human osteosarcoma cells - siRNA for CDK4

Lysates/proteins at 20 µg per lane.

Secondary

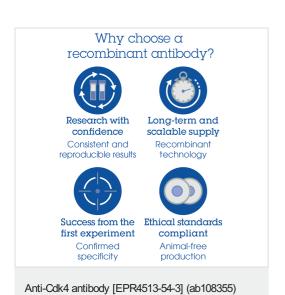
All lanes : HRP-conjugated goat anti-rabbit lgG polyclonal at 1/2000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 34 kDa **Observed band size:** 34 kDa

Exposure time: 5 seconds



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