

Product datasheet

HRP Anti-Cdk4 antibody [EPR4513-32-7] - Loading Control ab193968

KO VALIDATED Recombinant RabMAB

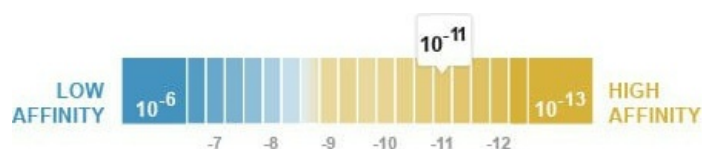
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Overview

Product name	HRP Anti-Cdk4 antibody [EPR4513-32-7] - Loading Control
Description	HRP Rabbit monoclonal [EPR4513-32-7] to Cdk4 - Loading Control
Host species	Rabbit
Conjugation	HRP
Tested applications	Suitable for: IHC-P, WB
Species reactivity	Reacts with: Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1, HeLa, MCF7, K562, and Ramos whole cell lysates. IHC-P: FFPE human breast adenocarcinoma.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAB[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAB[®] patents.</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle. Store In the Dark.
Dissociation constant (K_D)	K _D = 1.86 x 10 ⁻¹¹ M



[Learn more about K_D](#)

Storage buffer	pH: 7.40 Preservative: 0.1% Proclin 300 Solution Constituents: 30% Glycerol (glycerin, glycerine), 1% BSA, PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR4513-32-7
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab193968 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

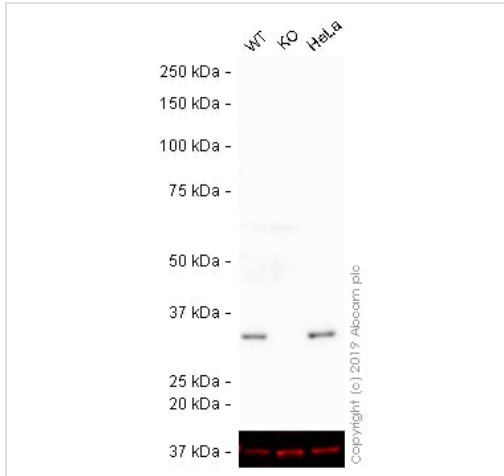
Application	Abreviews	Notes
IHC-P		1/500. Antigen retrieval is recommend.
WB		1/5000. Detects a band of approximately 34 kDa (predicted molecular weight: 34 kDa).

Target

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 mitogenic 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.
Involvement in disease	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.
Sequence similarities	Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. CDC2/CDKX subfamily. Contains 1 protein kinase domain.
Post-translational modifications	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.
Cellular localization	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.

Images



Western blot - HRP Anti-Cdk4 antibody [EPR4513-32-7] - Loading Control (ab193968)

All lanes : HRP Anti-Cdk4 antibody [EPR4513-32-7] - Loading Control (ab193968) at 1/5000 dilution

Lane 1 : Wild-type HAP1 cell lysate

Lane 2 : CDK4 knockout HAP1 cell lysate

Lane 3 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 34 kDa

Observed band size: 34 kDa

Exposure time: 30 seconds

ab193968 was shown to react with Cdk4 in wild-type HAP1 cells in Western blot. Loss of signal was observed when CDK4 knockout sample was used. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab193968 overnight at 4°C at a 1 in 5000 dilution and **ab184095** (Mouse Anti-GAPDH antibody [mAbcam 9484] - Alexa Fluor® 680) at a 1 in 1000 dilution. Blots were developed with Optiblot ECL reagent (**ab133456**) and imaged.



Western blot - HRP Anti-Cdk4 antibody [EPR4513-32-7] - Loading Control (ab193968)

All lanes : HRP Anti-Cdk4 antibody [EPR4513-32-7] - Loading Control (ab193968) at 1/5000 dilution

Lane 1 : HeLa whole cell lysate ([ab150035](#))

Lane 2 : MCF7 (Human breast adenocarcinoma cell line) Whole Cell Lysate

Lane 3 : K562 (Human erythromyeloblastoid leukemia cell line) Whole Cell Lysate

Lane 4 : Ramos (Human Burkitt's lymphoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Developed using the ECL technique.

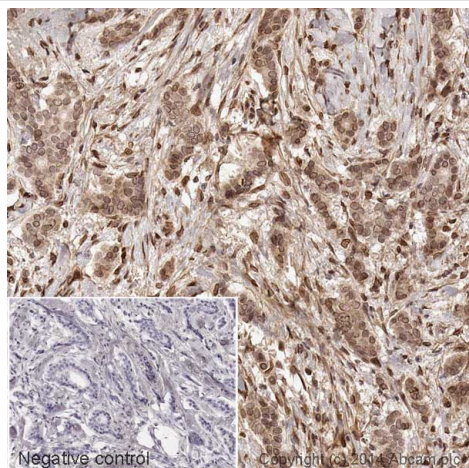
Performed under reducing conditions.

Predicted band size: 34 kDa

Observed band size: 34 kDa

Exposure time: 90 seconds

This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% milk before being incubated with ab193968 overnight at 4°C. Antibody binding was visualised using ECL development solution [ab133406](#).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - HRP Anti-Cdk4 antibody [EPR4513-32-7] - Loading Control (ab193968)

IHC image of Cdk4 staining in a section of formalin-fixed paraffin-embedded human breast adenocarcinoma*. The section was pre-treated using pressure cooker heat mediated antigen retrieval with sodium citrate buffer (pH6) for 30mins, and incubated overnight at +4°C with ab193968 at a working dilution of 1 in 500. DAB was used as the chromogen (**ab103723**), diluted 1/100 and incubated for 10min at room temperature. The section was counterstained with haematoxylin and mounted with DPX. The inset negative control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and manual) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre.

Why choose a recombinant antibody?



Research with confidence
Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

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Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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