# abcam

## Product datasheet

## Anti-Cdk4 antibody ab95255

## KO VALIDATED

## 1 References 2 Images

### Overview

Product name Anti-Cdk4 antibody

**Description** Rabbit polyclonal to Cdk4

Host species Rabbit

Tested applications Suitable for: WB

Species reactivity Reacts with: Human

Predicted to work with: Pig ...

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

**Positive control**This antibody gave a positive signal in both MCF7 and HeLa whole cell lysates.

General notes

The Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

## **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 or -

80°C. Avoid freeze / thaw cycle.

**Storage buffer** pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising

agent. If you would like information about the formulation of a specific lot, please contact our

scientific support team who will be happy to help.

**Purity** Immunogen affinity purified

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**Clonality** Polyclonal

**Isotype** IgG

### **Applications**

#### The Abpromise guarantee

Our Abpromise guarantee covers the use of ab95255 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
WB		Use a concentration of 1 µg/ml. Detects a band of approximately 33 kDa (predicted molecular weight: 33 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 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.

#### 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.

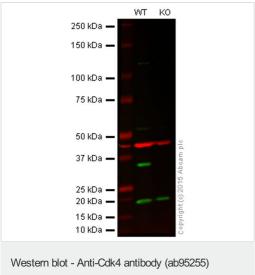
# Post-translational modifications

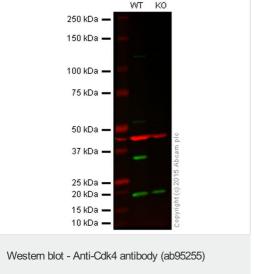
Phosphorylation at Thr-172 is required for enzymatic activity. Phosphorylated, in vitro, at this site by CCNH-CDK7, but, in vivo, appears to be phosphorylated by a proline-directed kinase. In the cyclin D-CDK4-CDKN1B complex, this phosphorylation and consequent CDK4 enzyme activity, is dependent on the 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.

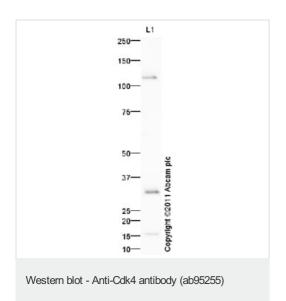
## Cellular localization

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**







**Lanes 1**: Wild-type HAP1 cell lysate (20 μg)

Lanes 2: CDK4 knockout HAP1 cell lysate (20 µg)

Lanes 1 and 2: Merged (red and green) signal. Green signal from target - ab95255 observed at 34 kDa. Red signal from loading control - ab8226 observed at 42 kDa

ab95255 was shown to react with CDK4 when CDK4 knockout samples were used, along with additional cross-reactive bands. Wild-type and CDK4 knockout samples were subjected to SDS-PAGE. ab95255 and ab8226 (loading control to beta actin) were diluted 1 µg/mL and 1/2000 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.

Anti-Cdk4 antibody (ab95255) at 1 µg/ml + MCF7 (Human breast adenocarcinoma cell line) Whole Cell Lysate at 10 µg

#### Secondary

Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 33 kDa Observed band size: 33 kDa

Additional bands at: 110 kDa, 16 kDa. We are unsure as to the

identity of these extra bands.

Exposure time: 20 minutes

A positive signal was also observed within HeLa whole cell lysate. Abcam recommends using milk as the blocking agent. Abcam welcomes customer feedback and would appreciate any comments regarding this product and the data presented above.

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

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