abcam

Product datasheet

Anti-Cyclin D2 antibody [EPR19659] ab207604

敲除验证 重组 RabMAb

1 Abreviews 21 References 10 图像

概述

产品名称	Anti-Cyclin D2抗体[EPR19659]
描述	兔单克隆抗体[EPR19659] to Cyclin D2
宿主	Rabbit
经测试应用	**适用于:** Flow Cyt (Intra), WB, ICC/IF, IP
种属反应性	**与反应:** Human
免疫原	Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: Full length human Cyclin D2 recombinant protein; HAP1, HEK-293, Caco-2 and U-2 OS whole cell lysates; human fetal brain, fetal heart and fetal kidney lysates. ICC/IF: Caco-2 and U-2 OS cells. IP: U-2 OS whole cell lysate. Flow Cyt (intra): U-2 OS 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 RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

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 long term. Avoid freeze / thaw cycle.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR19659

同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab207604于以下的经测试应用

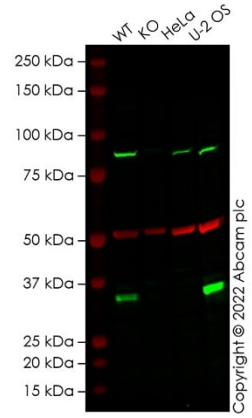
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/450.
WB		1/1000. Detects a band of approximately 33 kDa (predicted molecular weight: 33 kDa).
ICC/IF	★☆☆☆☆ (1)	1/100.
IP		1/30.

靶标

功能	Regulatory component of the cyclin D2-CDK4 (DC) complex that phosphorylates and inhibits members of the retinoblastoma (RB) protein family including RB1 and regulates the cell-cycle during G(1)/S transition. Phosphorylation of RB1 allows dissociation of the transcription factor E2F from the RB/E2F complex 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 substrate for SMAD3, phosphorylating SMAD3 in a cell-cycle-dependent manner and repressing its transcriptional activity. Component of the ternary complex, cyclin D2/CDK4/CDKN1B, required for nuclear translocation and activity of the cyclin D-CDK4 complex.
序列相似性	Belongs to the cyclin family. Cyclin D subfamily. Contains 1 cyclin N-terminal domain.
细胞定位	Nucleus. Cytoplasm. Membrane. Cyclin D-CDK4 complexes accumulate at the nuclear membrane and are then translocated into the nucleus through interaction with KIP/CIP family members.

图片



Western blot - Anti-Cyclin D2 antibody [EPR19659] (ab207604)

All lanes : Anti-Cyclin D2 antibody [EPR19659] (ab207604) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : Human CCND2 (Cyclin D2) knockout HEK-293T cell lysate (**ab257875**)
Lane 3 : HeLa cell lysate
Lane 4 : U-2 OS cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

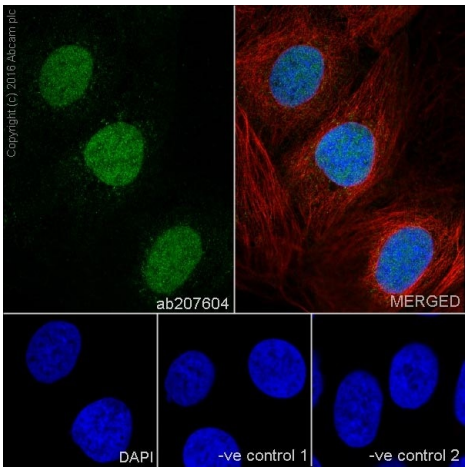
False colour image of Western blot: Anti-Cyclin D2 antibody [EPR19659] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab207604 was shown to bind specifically to Cyclin D2.

A band was observed at 33 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in Ccnd2 knockout cell line **ab267318** (knockout cell lysate **ab257875**). To generate this image, wild-type and Ccnd2 knockout HEK-293T cell lysates were analysed.

First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged.

Secondary antibodies used were Goat anti-Rabbit IgG H&L

(IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.

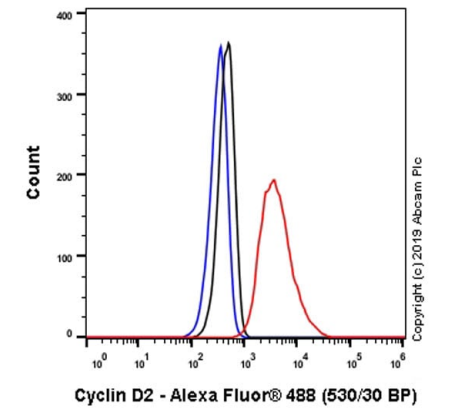


Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D2 antibody [EPR19659] (ab207604)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Caco-2 (Human colorectal adenocarcinoma cell line) cells labeling Cyclin D2 with ab207604 at 1/100 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear and weak cytoplasmic staining on Caco-2 cells. The nuclear counter stain is DAPI (blue).

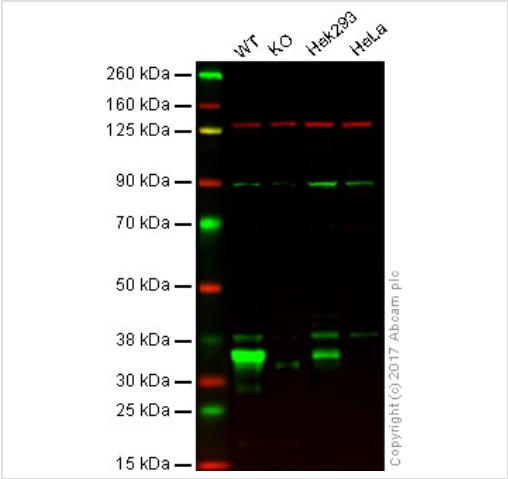
Tubulin is detected with Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:
-ve control 1: ab207604 at 1/100 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution.
-ve control 2: Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-Cyclin D2 antibody [EPR19659] (ab207604)

Intracellular Flow Cytometry analysis of U-2 OS (Human bone osteosarcoma epithelial cell) cells labeling Cyclin D2 with purified ab207604 at 1/450 dilution (1.00µg/mL) (Red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (**ab172730**) (black). Unlabeled control - Unlabelled cells (blue).

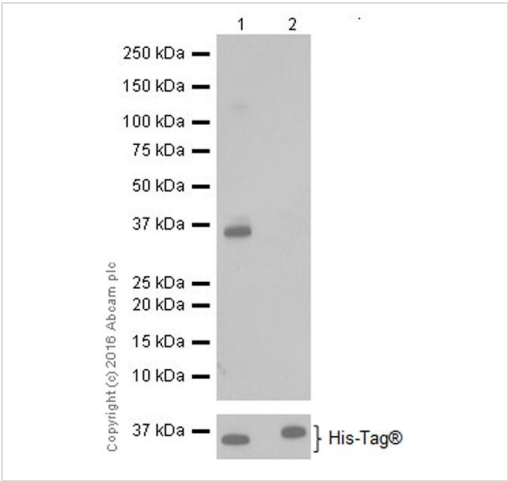


Western blot - Anti-Cyclin D2 antibody [EPR19659] (ab207604)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: CCND2 (Cyclin D2) knockout HAP1 whole cell lysate (20 µg)
Lane 3: Hek293 whole cell lysate (20 µg)
Lane 4: HeLa whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab207604 observed at 34 kDa. Red - loading control, **ab18058**, observed at 130 kDa.

ab207604 was shown to recognize CCND2 (Cyclin D2) in wild type cells as signal was lost at the expected MW in CCND2 (Cyclin D2) knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and CCND2 (Cyclin D2) knockout samples were subjected to SDS-PAGE. Ab207604 and **ab18058** (Mouse anti Vinculin loading control) were incubated overnight at 4°C at a 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Cyclin D2 antibody [EPR19659] (ab207604)

All lanes : Anti-Cyclin D2 antibody [EPR19659] (ab207604) at 1/1000 dilution

Lane 1 : Full length human Cyclin D2 recombinant protein
Lane 2 : Full length human Cyclin D1 recombinant protein

Lysates/proteins at 0.01 µg per lane.

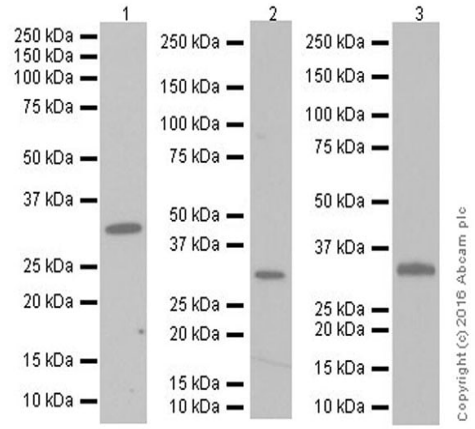
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 33 kDa
Observed band size: 35 kDa

Exposure time: 3 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

Full length human Cyclin D2 recombinant protein contains aa1-289 with a His-Tag. Full length human Cyclin D1 recombinant protein contains aa1-295 with a His-Tag. These two recombinant proteins were made in-house.



Western blot - Anti-Cyclin D2 antibody [EPR19659] (ab207604)

All lanes : Anti-Cyclin D2 antibody [EPR19659] (ab207604) at 1/1000 dilution

Lane 1 : HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 2 : Caco-2 (Human colorectal adenocarcinoma cell line) whole cell lysate
Lane 3 : U-2 OS (Human bone osteosarcoma epithelial cell line) whole cell lysate

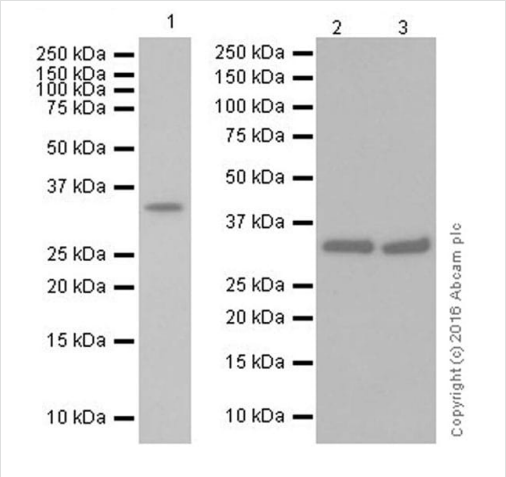
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Cyclin D2 antibody [EPR19659] (ab207604)

All lanes : Anti-Cyclin D2 antibody [EPR19659] (ab207604) at 1/1000 dilution

Lane 1 : Human fetal brain lysate
Lane 2 : Human fetal heart lysate
Lane 3 : Human fetal kidney lysate

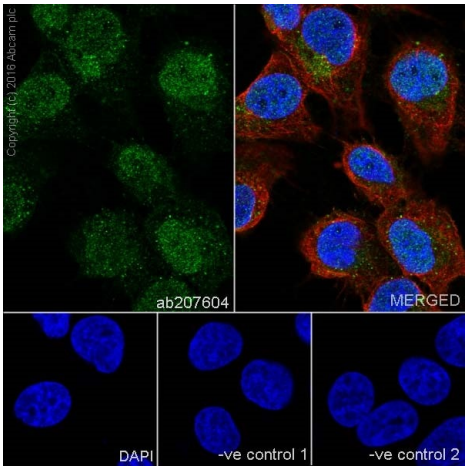
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

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

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

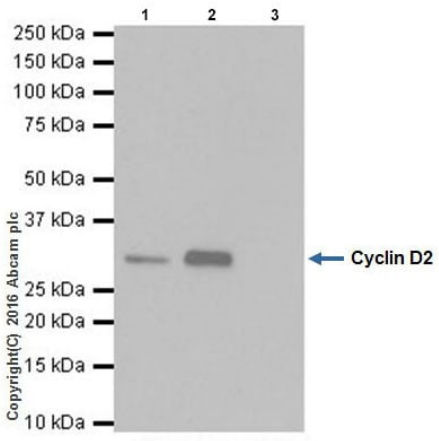


Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D2 antibody [EPR19659] (ab207604)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized U-2 OS (Human bone osteosarcoma epithelial cell line) cells labeling Cyclin D2 with ab207604 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear and weak cytoplasmic staining on U-2 OS cells. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:
-ve control 1: ab207604 at 1/100 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution.
-ve control 2: Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary at 1/1000 dilution.



Immunoprecipitation - Anti-Cyclin D2 antibody [EPR19659] (ab207604)

Cyclin D2 was immunoprecipitated from 0.35 mg of U-2 OS (Human bone osteosarcoma epithelial cell line) whole cell lysate with ab207604 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab207604 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

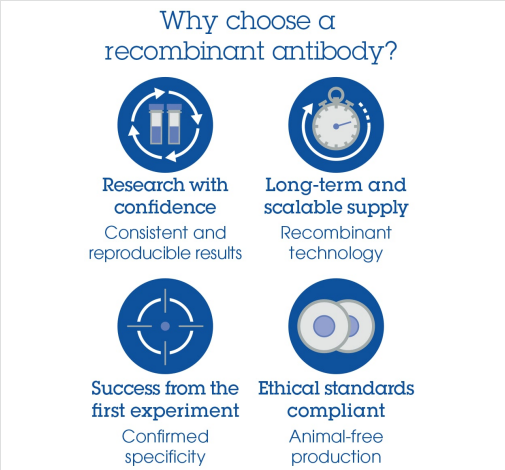
Lane 1: U-2 OS whole cell lysate, 10µg (Input).

Lane 2: ab207604 IP in U-2 OS whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab207604 in U-2 OS whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes.



Anti-Cyclin D2 antibody [EPR19659] (ab207604)

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

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.cn/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors