abcam

Product datasheet

Anti-Cyclin D2 antibody [EPR19659] - BSA and Azide free ab240377

KO VALIDATED Recombinant RabMAb

7 Images

Overview

Product name	Anti-Cyclin D2 antibody [EPR19659] - BSA and Azide free
Description	Rabbit monoclonal [EPR19659] to Cyclin D2 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, Flow Cyt (Intra), ICC/IF, IP
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Wild type HEK293T, HAP1 and HeLa whole cell lysate. Flow Cyt (intra): U-2 OS cells ICC/IF: Caco-2 and U-2 OS cells. IP: U-2 OS cell lysate.
General notes	ab240377 is the carrier-free version of **ab207604**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR19659
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab240377 in the following tested applications.

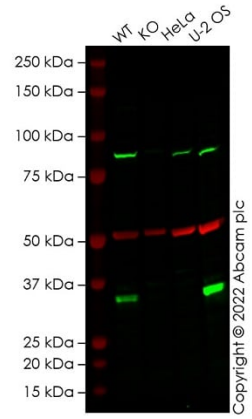
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 33 kDa (predicted molecular weight: 33 kDa).
Flow Cyt (Intra)		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

Target

Function	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.
Sequence similarities	Belongs to the cyclin family. Cyclin D subfamily. Contains 1 cyclin N-terminal domain.
Cellular localization	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.

Images



Western blot - Anti-Cyclin D2 antibody [EPR19659] - BSA and Azide free (ab240377)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab207604**).

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.

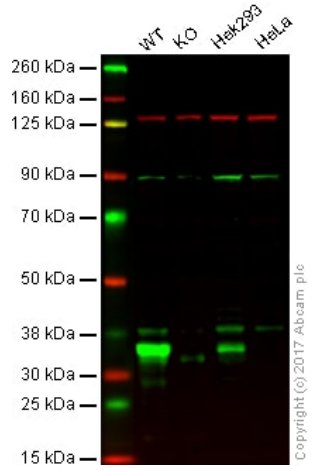
A band was observed at 33 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in Ccnd2 knockout cell line **ab267318** (knockout cell lysate **ab257875**). To generate this image, wild-type and Ccnd2 knockout HEK-293T cell lysates were analysed.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab207604**).



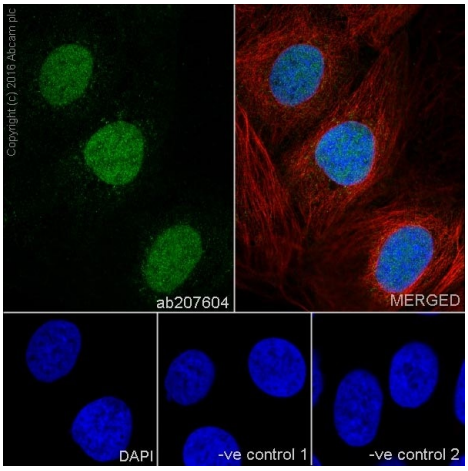
Western blot - Anti-Cyclin D2 antibody [EPR19659] - BSA and Azide free (ab240377)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab207604**).



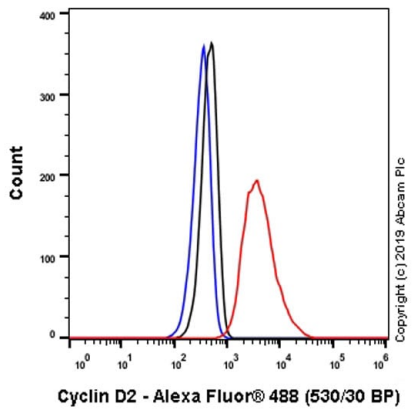
Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D2 antibody [EPR19659] - BSA and Azide free (ab240377)

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).

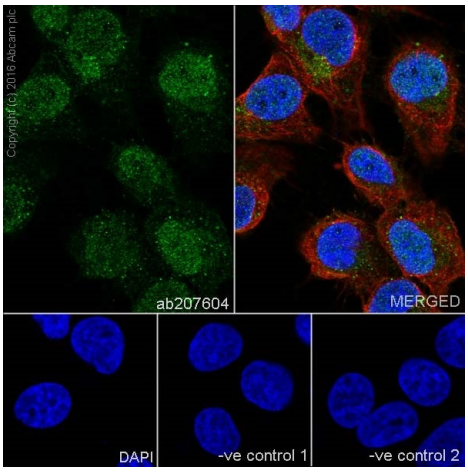
The negative controls are as follows:
-ve control 1: **ab207604** at 1/100 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution.
-ve control 2: Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab207604**).



Flow Cytometry (Intracellular) - Anti-Cyclin D2 antibody [EPR19659] - BSA and Azide free (ab240377)

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). This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab207604**).



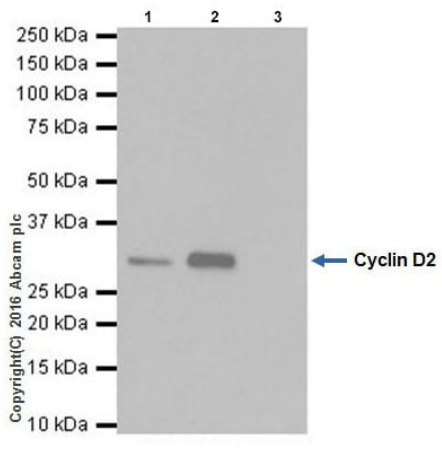
Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D2 antibody [EPR19659] - BSA and Azide free (ab240377)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab207604**).



Immunoprecipitation - Anti-Cyclin D2 antibody [EPR19659] - BSA and Azide free (ab240377)

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.

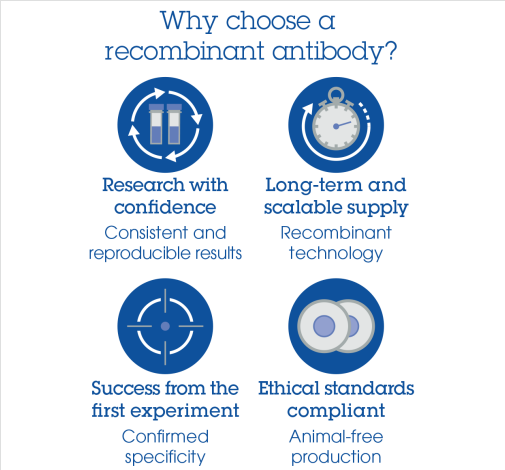
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab207604** in U-2 OS whole cell lysate.

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

Exposure time: 3 minutes.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and

sodium azide (**ab207604**).



Anti-Cyclin D2 antibody [EPR19659] - BSA and Azide free (ab240377)

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