abcam

ab207604

Anti-Cyclin D2 antibody [EPR19659]

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR19659
Purification technique	Affinity purification Protein A
Concentration	0.483 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Not recommended

Species	Recombinant full length protein - Human
Dilution info	-
Notes	-

WB

Tested

Species	Recombinant full length protein - Human
Dilution info	1/1000
Notes	-

Species	Human
Dilution info	1/1000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100
Notes	-

Not recommended

Species	Recombinant full length protein - Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/450

Notes	-

Not recommended

Species	Recombinant full length protein - Human
Dilution info	-
Notes	-

Target data

See full target information CCND2

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 (PubMed:18827403, PubMed:8114739). 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 (PubMed:18827403, PubMed:8114739). Hypophosphorylates RB1 in early G(1) phase (PubMed:18827403, PubMed:8114739). Cyclin D-CDK4 complexes are major integrators of various mitogenenic and antimitogenic signals (PubMed:18827403, PubMed:8114739).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For

details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

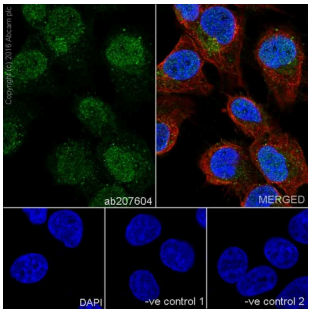
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

10 product images



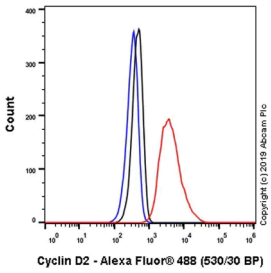
Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D2 antibody [EPR19659] (ab207604)

Cyclin D2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Cyclin D2 antibody

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).

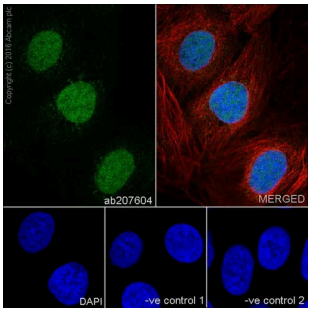
The negative controls are as follows:
-ve control 1: ab207604 at 1/100 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 1/1000 dilution.
-ve control 2: Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa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



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

Cyclin D2 Flow Cytometry (Intracellular) staining using rabbit Anti-Cyclin D2 antibody

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).

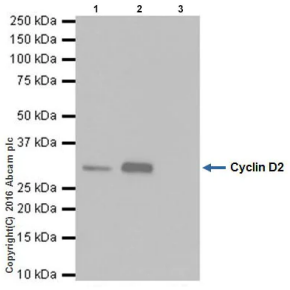


Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D2 antibody [EPR19659] (ab207604)

Cyclin D2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Cyclin D2 antibody

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.



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).

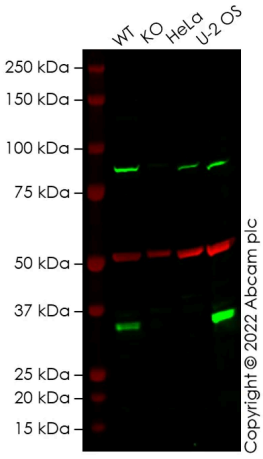
Lane 2: ab207604 IP in U-2 OS whole cell lysate.

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.
All lanes:
Immunoprecipitation - Anti-Cyclin D2 antibody [EPR19659] (ab207604)

Predicted band size: 30 kDa, 33 kDa



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

Lane 1:
Wild-type HEK-293T cell lysate at 20 µg

Lane 2:
Western blot - Human CCND2 (Cyclin D2) knockout HEK-293T cell lysate (ab257875) at 20 µg

Lane 2:
Western blot - Human CCND2 (Cyclin D2) knockout HEK-293T cell lysate (ab257875)

Lane 2:
Western blot - Human CCND2 (Cyclin D2) knockout HEK-293T cell line (ab267318)

Lane 3:
HeLa cell lysate at 20 µg

Lane 4:
U-2 OS cell lysate at 20 µg

Secondary

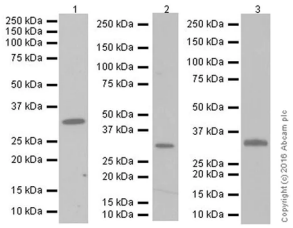
Lanes 1 - 4:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/20000 dilution

Lanes 1 - 4:
Western blot - Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 33 kDa

Observed band size: 33 kDa



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

Cyclin D2 Western blot staining using rabbit Anti-Cyclin D2 antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-Cyclin D2 antibody [EPR19659] (ab207604) at 1/1000 dilution

Lane 1:
HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate at 10 µg

Lane 2:
Caco-2 (Human colorectal adenocarcinoma cell line) whole cell lysate at 10 µg

Lane 3:
U-2 OS (Human bone osteosarcoma epithelial cell line) whole cell lysate at 10 µg

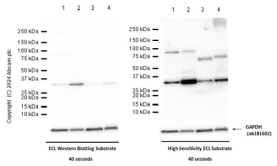
Secondary

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

Predicted band size: 33 kDa

Observed band size: 33 kDa

Exposure time: 3min



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

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

ab181602 was used as a GAPDH loading control.

We recommend using a higher sensitive ECL substrate to increase the band intensity.

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

Lane 1:
293T (Human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Lane 2:
U-2 OS (Human bone osteosarcoma epithelial cell) whole cell lysate at 20 µg

Lane 3:
Human heart tissue lysate at 20 µg

Lane 4:
Human thyroid tissue lysate at 20 µg

Secondary

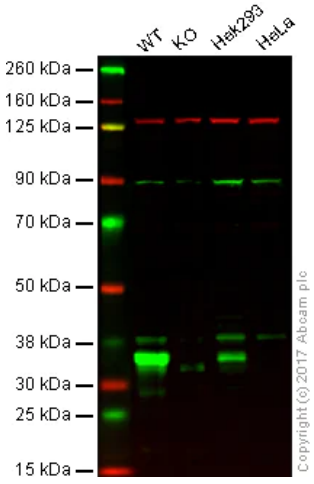
All lanes:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Developed using the ECL technique.

Predicted band size: 33 kDa

Observed band size: 31 kDa

Exposure time: 40s



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

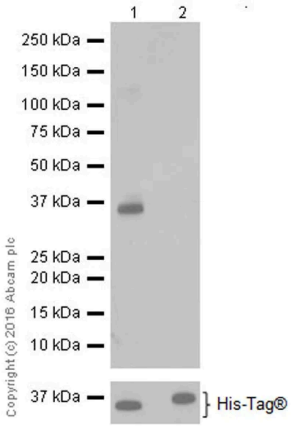
Cyclin D2 Western blot staining using rabbit Anti-Cyclin D2 antibody

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.

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

Predicted band size: 33 kDa



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

Cyclin D2 Western blot staining using rabbit Anti-Cyclin D2 antibody

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.

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

Lane 1:
Full length human Cyclin D2 recombinant protein at 0.01 µg

Lane 2:
Full length human Cyclin D1 recombinant protein at 0.01 µg

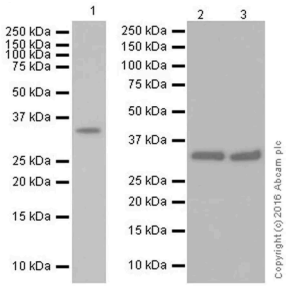
Secondary

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

Predicted band size: 33 kDa

Observed band size: 35 kDa

Exposure time: 3s



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

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-Cyclin D2 antibody [EPR19659] (ab207604) at 1/1000 dilution

Lane 1:
Human fetal brain lysate at 10 µg

Lane 2:
Human fetal heart lysate at 10 µg

Lane 3:
Human fetal kidney lysate at 10 µg

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: 3min

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.