abcam

ab16663

Anti-Cyclin D1 antibody [SP4]

Anti-Cyclin D1 antibody [SP4] (ab16663) is a rabbit monoclonal antibody detecting Cyclin D1 in **Western Blot, Flow Cytometry (Intra), IHC-P, ICC/IF**. Suitable for **Human, Mouse, Rat**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 550 publications
- Trusted since 2005

Lab Essentials | Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.1% Sodium azide Constituents: PBS, 1% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	SP4
Purification technique	Affinity purification Protein A
Epitope	C-terminus
Concentration	0.046 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Rat
Dilution info	1/100
Notes	Deparaffinization: Deparaffinize slides using xylene or xylene alternative and graded alcohols. Antigen Retrieval: Boil tissue section in 10mM citrate buffer, pH 6.0 for 10 min followed by cooling at room temperature for 20 min. Primary Antibody Incubation: Incubate for 30 minutes at room temperature. Slide Washing: Slides must be washed in between steps. Rinse slides with PBS/0.05% Tween. Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Species	Human
Dilution info	1/100
Notes	Deparaffinization: Deparaffinize slides using xylene or xylene alternative and graded alcohols. Antigen Retrieval: Boil tissue section in 10mM citrate buffer, pH 6.0 for 10 min followed by cooling at room temperature for 20 min. Primary Antibody Incubation: Incubate for 30 minutes at room temperature. Slide Washing: Slides must be washed in between steps. Rinse slides with PBS/0.05% Tween. Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Human
Dilution info	1/25 - 1/200
Notes	-

Species	Mouse
Dilution info	1/25 - 1/200

Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	1/50 - 1/250
Notes	-

Species	Rat
Dilution info	1/50 - 1/250
Notes	-

Species	Human
Dilution info	1/50 - 1/250
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	1/30
Notes	-

Species	Rat
Dilution info	1/30
Notes	-

Species	Human
Dilution info	1/30
Notes	-

Target data

See full target information CCND1

Function	Regulatory component of the cyclin D1-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:1827756, PubMed:1833066, PubMed:19412162, PubMed:33854235, PubMed:8114739, PubMed:8302605). 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:1827756, PubMed:1833066, PubMed:19412162, PubMed:8114739, PubMed:8302605). Hypophosphorylates RB1 in early G(1) phase (PubMed:1827756, PubMed:1833066, PubMed:19412162, PubMed:8114739, PubMed:8302605). Cyclin D-CDK4 complexes are major integrators of various mitogenenic and antimitogenic signals (PubMed:1827756, PubMed:1833066, PubMed:19412162, PubMed:8302605). Also a substrate for SMAD3, phosphorylating SMAD3 in a cell-cycle-dependent manner and repressing its transcriptional activity (PubMed:15241418). Component of the ternary complex, cyclin D1/CDK4/CDKN1B, required for nuclear translocation and activity of the cyclin D-CDK4 complex (PubMed:9106657). Exhibits transcriptional corepressor activity with INSM1 on the NEUROD1 and INS promoters in a cell cycle-independent manner (PubMed:16569215, PubMed:18417529).

Storage

Shipped at conditions	Conditional Ambient
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

This product was switched from a hybridoma to recombinant production method on 22nd January 2019.

Product Specifications
Anti-Cyclin D1 antibody [SP4] (ab16663) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt (Intra), ICC/IF, IHC-P, WB in human, mouse, rat samples.
Anti-Cyclin D1 antibody [SP4] (ab16663) specifically detects Cyclin D1 (UniProt ID: P24385; Molecular weight: 34kDa) and is sold in 100 µL, 500 µL and 1 mL selling sizes.
Anti-Cyclin D1 antibody [SP4] (ab16663) is a clone from the portfolio of Spring Bioscience (Roche) SP clones which

have been optimised for immunohistochemistry (IHC).

Quality and Validation
Abcam's high quality manufacturing and validation processes ensure Anti-Cyclin D1 antibody [SP4] (ab16663) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.
The specificity of Anti-Cyclin D1 antibody [SP4] (ab16663) has been confirmed by testing in knockout samples.
Anti-Cyclin D1 antibody [SP4] (ab16663) has been cited over 559 times in peer reviewed journals and is trusted by the scientific community.
Anti-Cyclin D1 antibody [SP4] (ab16663) has 23 independent reviews from customers.

Related Products
Antibody clone SP4 is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor® 647 (ab267515).
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

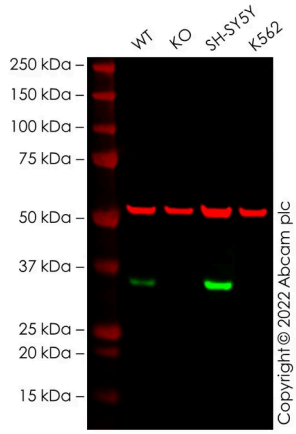
We do not recommend this combination. It is not covered by our product promise.

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.

23 product images



Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663)

Cyclin D1 Western blot staining using rabbit Anti-Cyclin D1 antibody

False colour image of Western blot: Anti-Cyclin D1 antibody [SP4] staining at 1/25 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab16663 was shown to bind specifically to Cyclin D1. A band was observed at 35 kDa in wild-type A549 cell lysates with no signal observed at this size in ccnd1 knockout cell line ab286759 (knockout cell lysate ab300213). To generate this image, wild-type and ccnd1 knockout A549 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 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663) at 1/25 dilution

Lane 1:
Wild-type A549 cell lysate at 20 µg

Lane 3:
SH-SY5Y cell lysate at 20 µg

Lane 4:
K562 cell lysate at 20 µg

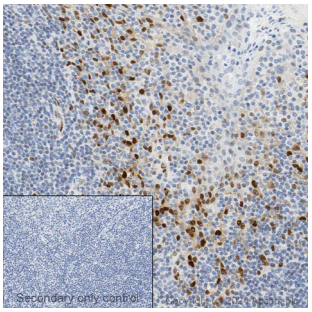
Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 34 kDa

Observed band size: 35 kDa



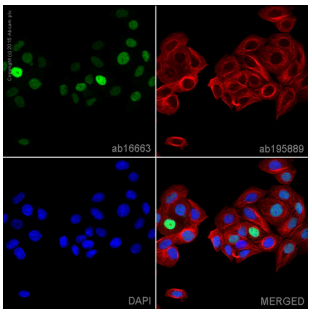
Immunohistochemistry - Anti-Cyclin D1 antibody [SP4] (ab16663)

Immunohistochemical analysis of formalin fixed paraffin embedded human tonsil labelling CyclinD1 with ab16663 at a dilution of 1/200.

The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was conducted for 32min with DISCOVERY cell conditioning solution (CC1) 100°C, pH8.5. ab16663 CyclinD1 was incubated at 37°C for 16min.

Sections were counterstained is with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

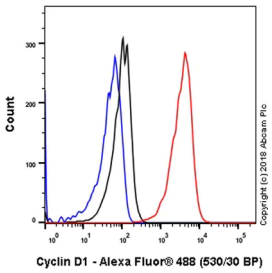
Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual)



Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D1 antibody [SP4] (ab16663)

Cyclin D1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Cyclin D1 antibody

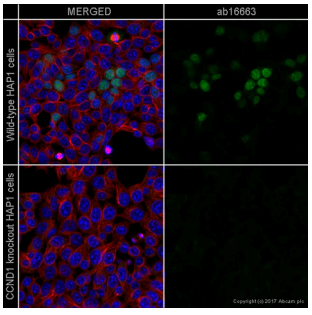
ab16663 staining Cyclin D1 in MCF7 cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab16663 at a working dilution of 1/250 and ab195889, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor® 594, shown in red) at 1/250 overnight at +4°C, followed by a further incubation at room temperature for 1h with an anti-rabbit AlexaFluor® 488 (ab150081) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).
This image was generated from the hybridoma version.



Flow Cytometry (Intracellular) - Anti-Cyclin D1 antibody [SP4] (ab16663)

Cyclin D1 Flow Cytometry (Intracellular) staining using rabbit Anti-Cyclin D1 antibody

Intracellular flow cytometry analysis of MCF-7 (human breast carcinoma) labeling Cyclin D1 with purified ab16663 at 1/30 dilution (9.03 µg/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as a secondary antibody. Isotype control - Rabbit monoclonal IgG (ab172730) (Black). Unlableled control - Unlabelled cells (blue). This image was generated from the hybridoma version.

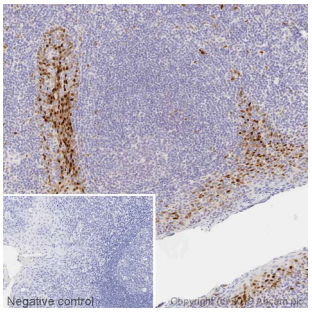


Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D1 antibody [SP4] (ab16663)

Cyclin D1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Cyclin D1 antibody

ab16663 staining Cyclin D1 in wild-type HAP1 cells (top panel) and CCND1 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab16663 at 1/250 dilution and ab195889 at 1/250 dilution (shown in pseudocolour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This image was generated from the hybridoma version.



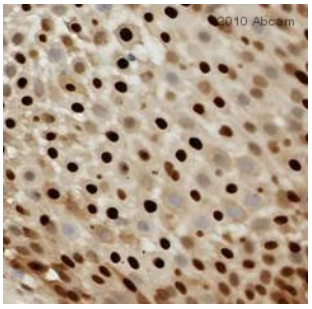
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin D1 antibody [SP4] (ab16663)

Cyclin D1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Cyclin D1 antibody

IHC image of ab16663 staining Cyclin D1 in a section of formalin-fixed paraffin-embedded normal human tonsil* performed on a Leica BOND^{TM} system using the standard protocol **F**. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab16663, 1/100 dilution, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.
For other IHC staining systems (automated and non-automated) 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*

This image was generated from the hybridoma version.

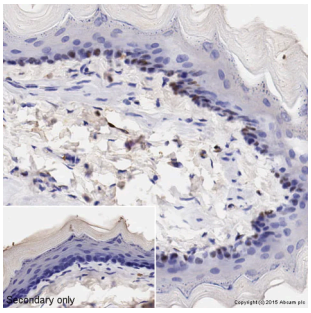


This image is courtesy of a customer review submitted by Karin Birkenkamp-Demtroeder

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin D1 antibody [SP4] (ab16663)

Cyclin D1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Cyclin D1 antibody

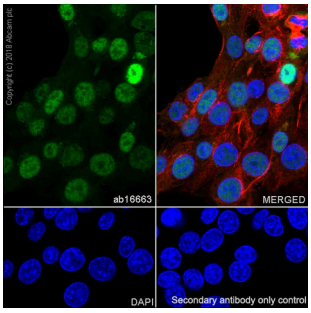
ab16663 staining Cyclin D1 in Human urinary tract tissue sections by Immunohistochemistry (IHC-P - formaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 10% BSA for 30 minutes at room temperature; antigen retrieval was by heat mediation in citrate buffer. Samples were incubated with primary antibody (1/100 in PBS) for 1 hour. An undiluted HRP-conjugated Goat anti-rabbit IgG polyclonal was used as the secondary antibody.
This image was generated from the hybridoma version.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin D1 antibody [SP4] (ab16663)

Cyclin D1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Cyclin D1 antibody

IHC image of ab16663 staining Cyclin D1 in rat esophagus formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab16663, 1:100 dilution, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. No primary antibody was used in the secondary only control (shown on the inset).
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.
This image was generated from the hybridoma version.



Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D1 antibody [SP4] (ab16663)

Cyclin D1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Cyclin D1 antibody

Immunocytochemistry/ Immunofluorescence analysis of C6 (rat glial tumor glial cell) cells labeling Cyclin D1 with purified ab16663 at 1/50 (5.42µg/ml). Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Cells were counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/1000 (2 µg/ml) dilution. DAPI was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.
This image was generated from the hybridoma version.

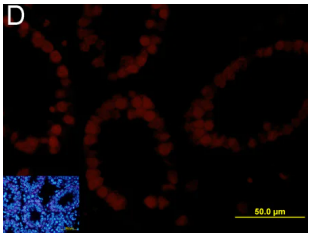


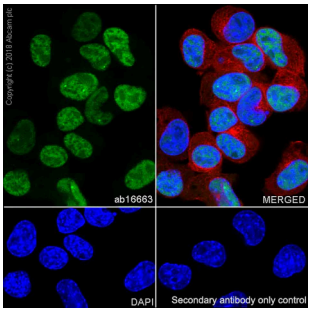
Image from McIver SC et al., PLoS One. 2012;7(4):e35553. Epub 2012 Apr 20. Fig 7.; doi:10.1371/journal.pone.0035553; April 20, 2012, PLoS ONE 7(4): e35553.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin D1 antibody [SP4] (ab16663)

Cyclin D1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Cyclin D1 antibody

Immunohistochemical analysis of mouse testis tissue, staining Cyclin D1 with ab16663.

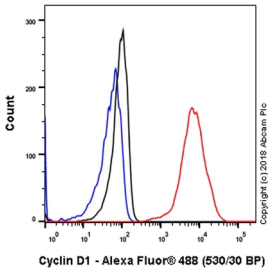
Antigen retrieval was performed via Tris-EDTA buffer. Sections were blocked with 3% BSA and incubated with primary antibody (1/50) overnight at 4°C. An AlexaFluor®594-conjugated secondary antibody was used to detect staining.
This image was generated from the hybridoma version.



Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D1 antibody [SP4] (ab16663)

Cyclin D1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Cyclin D1 antibody

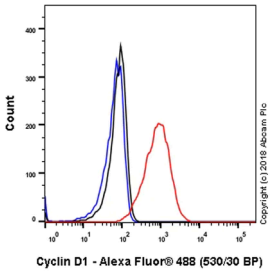
Immunocytochemistry/ Immunofluorescence analysis of Neuro-2a (mouse neuroblastoma neuroblast) cells labeling Cyclin D1µ with purified ab16663 at 1/50 (5.42µg/ml). Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Cells were counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/1000 (2 µg/ml) dilution. DAPI was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.
This image was generated from the hybridoma version.



Flow Cytometry (Intracellular) - Anti-Cyclin D1 antibody [SP4] (ab16663)

Cyclin D1 Flow Cytometry (Intracellular) staining using rabbit Anti-Cyclin D1 antibody

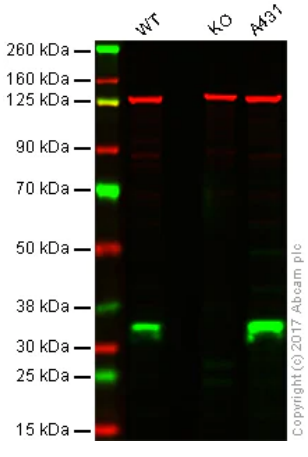
Intracellular flow cytometry analysis of NIH/3T3 (mouse embryo) labeling Cyclin D1 with purified ab16663 at 1/30 dilution (9.03 µg/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as a secondary antibody. Isotype control - Rabbit monoclonal IgG (ab172730) (Black). Unlableled control - Unlabelled cells (blue). This image was generated from the hybridoma version.



Flow Cytometry (Intracellular) - Anti-Cyclin D1 antibody [SP4] (ab16663)

Cyclin D1 Flow Cytometry (Intracellular) staining using rabbit Anti-Cyclin D1 antibody

Intracellular flow cytometry analysis of C6 (rat glioma) labeling Cyclin D1 with purified ab16663 at 1/30 dilution (9.03 µg/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as a secondary antibody. Isotype control - Rabbit monoclonal IgG (ab172730) (Black). Unlableled control - Unlabelled cells (blue). This image was generated from the hybridoma version.



Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663)

Cyclin D1 Western blot staining using rabbit Anti-Cyclin D1 antibody

Lanes 1 - 3: Merged signal (red and green). Green - ab16663 observed at 34 kDa. Red - loading control, ab18058, observed at 130 kDa.
ab16663 was shown to specifically recognize CCND1 (Cyclin D1) in wild-type HAP1 cells as signal was lost at the expected MW in CCND1 (Cyclin D1) knockout cells. Wild-type and CCND1 (Cyclin D1) knockout samples were subjected to SDS-PAGE. ab16663 and ab18058 (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 1/200 dilution and 1/20,000 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 1/20,000 dilution for 1 hour at room temperature before imaging.

This image was generated from the hybridoma version.

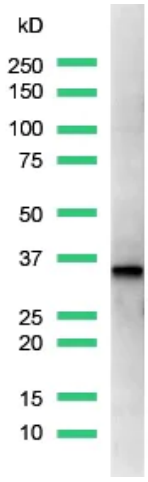
All lanes:
Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663)

Lane 1:
Wild-type HAP1 whole cell lysate at 20 µg

Lane 2:
CCND1 (Cyclin D1) knockout HAP1 whole cell lysate at 20 µg

Lane 3:
A431 whole cell lysate at 20 µg

Predicted band size: 34 kDa



Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663)

This image was generated from the hybridoma version.
All lanes:
Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663) at 1/25 dilution

All lanes:
MCF7 cell lysate

Predicted band size: 34 kDa

Observed band size: 36 kDa

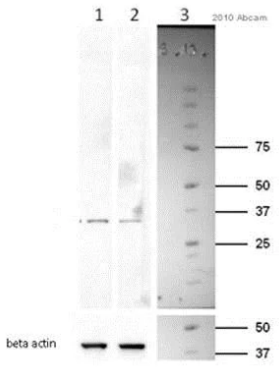


Image kindly supplied by Dr Karin Birkenkamp-Demtroeder through customer review

Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663)

Gel run under denaturing conditions 4-12% gradient.
This image was generated from the hybridoma version.

Lane 1:
Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663) at 1/200 dilution

Lane 2:
Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663) at 1/400 dilution

All lanes:
Whole cell lysate prepared from T24 bladder cancer cells at 25 µg

Secondary

All lanes:
Goat anti-rabbit IgG conjugated to HRP at 1/5000 dilution

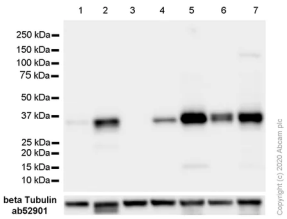
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 34 kDa

Observed band size: 33 kDa

Exposure time: 10min



Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663)

All lanes:
Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663) at 1/1000 dilution

Lane 1:
HeLa (Human cervix adenocarcinoma epithelial cell) cell lysate at 10 µg

Lane 2:
MCF7 (Human breast adenocarcinoma epithelial cell) cell lysate at 10 µg

Lane 3:
CCND1 KO HAP1 cell lysate at 10 µg

Lane 4:
HAP1 (Human chronic myelogenous leukemia near-haploid cell line) cell lysate at 10 µg

Lane 5:
Neuro-2a (Mouse neuroblastoma neuroblast) cell lysate at 10 µg

Lane 6:
NIH/3T3 (Mouse embryonic fibroblast) cell lysate at 10 µg

Lane 7:
C6 (Rat glial tumor glial cell) cell lysate at 10 µg

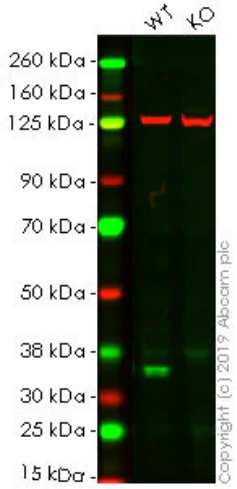
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 34 kDa

Observed band size: 33 kDa

Exposure time: 2s



Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663)

Cyclin D1 Western blot staining using rabbit Anti-Cyclin D1 antibody

Lanes 1- 2: Merged signal (red and green). Green - ab16663 observed at 36 kDa. Red - Anti-Vinculin antibody [VIN-54] observed at 124 kDa.
ab16663 was shown to react with CCND1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab255348 (knockout cell lysate ab263808) was used. Wild-type HeLa and CCND1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab16663 and Anti-Vinculin antibody [VIN-54] overnight at 4°C at a 1 in 200 dilution and a 1 in 20000 dilution respectively. 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 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663) at 1/200 dilution

Lane 1:
Wild-type HeLa cell lysate at 20 µg

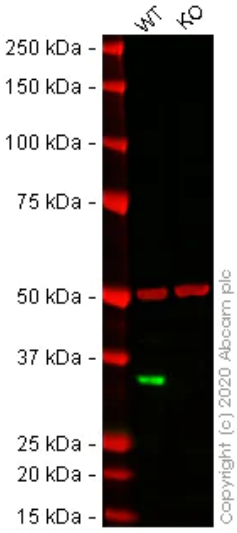
Lane 2:
CCND1 knockout HeLa cell lysate at 20 µg

Lane 2:
Western blot - Human CCND1 (Cyclin D1) knockout HeLa cell line (ab255348)

Performed under reducing conditions.

Predicted band size: 34 kDa

Observed band size: 36 kDa



Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663)

Cyclin D1 Western blot staining using rabbit Anti-Cyclin D1 antibody

Lanes 1- 2: Merged signal (red and green). Green - ab16663 observed at 36 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) observed at 50 kDa.
ab16663 was shown to react with Cyclin D1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab261760 (knockout cell lysate ab256864) was used. Wild-type HeLa and CCND1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab16663 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) overnight at 4°C at a 1 in 200 dilution and a 1 in 20000 dilution respectively. 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 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663) at 1/200 dilution

Lane 1:
Wild-type HeLa cell lysate at 20 µg

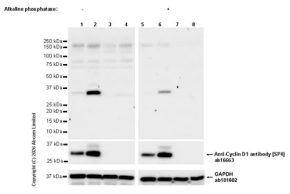
Lane 2:
CCND1 knockout HeLa cell lysate at 20 µg

Lane 2:
Western blot - Human CCND1 (Cyclin D1) knockout HeLa cell line (ab261760)

Performed under reducing conditions.

Predicted band size: 34 kDa

Observed band size: 36 kDa



Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663)

Cyclin D1 Western blot staining using rabbit Anti-Cyclin D1 antibody

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

Performed under reducing conditions.

In Western blot, aab325926 was shown to bind specifically to Cyclin D1 (phospho T286). Target of interest was observed at 33 kDa in A549 treated with 10µM MG-132 for 4 hours lysates (lane 2) with no signal observed at this size in CCND1 (Cyclin D1) knockout cell line (lanes 3-4) (lanes 3-4, knockout cell line ab286759).

The identity of the bands higher than 75kda are unknown.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) (1:200000) (36KDa).
All lanes:
Western blot - Anti-Cyclin D1 (phospho T286) antibody [EPR30260-527] (ab325926) at 1/1000 dilution

Lane 1:
Untreated A549 (human lung carcinoma epithelial cell) whole cell lysate 20 µg (untreated membrane)

Lane 2:
A549 treated with 10µM MG-132 for 4 hours whole cell lysate 20 µg (untreated membrane)

Lane 3:
Untreated CCND1 (Cyclin D1) knockout A549 whole cell lysate 20 µg (untreated membrane)

Lane 4:
CCND1 (Cyclin D1) knockout A549 treated with 10µM MG-132 for 4 hours whole cell lysate 20 µg (untreated membrane)

Lane 5:
Untreated A549 (human lung carcinoma epithelial cell) whole cell lysate 20 µg (alkaline phosphatase treated membrane)

Lane 6:
A549 treated with 10µM MG-132 for 4 hours whole cell lysate 20 µg (alkaline phosphatase treated membrane)

Lane 7:
Untreated CCND1 (Cyclin D1) knockout A549 whole cell lysate 20 µg (alkaline phosphatase treated membrane)

Lane 8:
CCND1 (Cyclin D1) knockout A549 treated with 10µM MG-132 for 4 hours whole cell lysate 20 µg (alkaline phosphatase treated membrane)

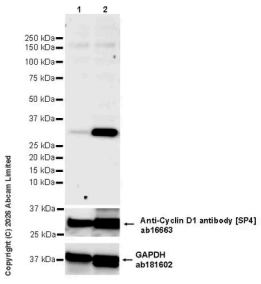
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Performed under reducing conditions.

Observed band size: 33 kDa, 36 kDa

Exposure time: 48s



Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663)

Cyclin D1 Western blot staining of HT-1080 treated with 10µM MG-132 for 4 hours whole cell lysate using rabbit Anti-Cyclin D1 antibody

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

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) (1:200000) (36KDa).

All lanes:
Western blot - Anti-Cyclin D1 (phospho T286) antibody [EPR30260-527] (ab325926) at 1/1000 dilution

Lane 1:
Untreated HT-1080 (human fibrosarcoma epithelial cell) whole cell lysate at 20 µg

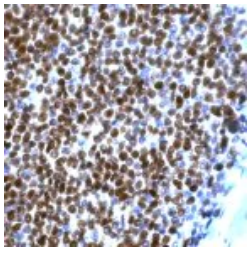
Lane 2:
HT-1080 treated with 10µM MG-132 for 4 hours whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 33 kDa, 36 kDa

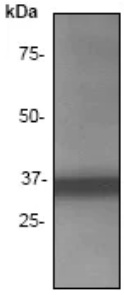
Exposure time: 92s



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin D1 antibody [SP4] (ab16663)

Cyclin D1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Cyclin D1 antibody

Human mantle cell lymphoma stained with ab16663.
This image was generated from the hybridoma version.



Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663)

Cyclin D1 Western blot staining of MCF-7 cell lysate using rabbit Anti-Cyclin D1 antibody

All lanes:
Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663) at 1/5000 dilution

All lanes:
MCF-7 cell lysate

Predicted band size: 34 kDa

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