

## Product datasheet

### Anti-Cyclin D1 antibody [SP4] ab16663

KO VALIDATED

Recombinant

RabMAb

★★★★☆ 23 Abreviews 487 References 21 Images

#### Overview

Product name	Anti-Cyclin D1 antibody [SP4]
Description	Rabbit monoclonal [SP4] to Cyclin D1
Host species	Rabbit
Tested applications	<b>Suitable for:</b> ICC/IF, Flow Cyt (Intra), WB, IHC-P
Species reactivity	<b>Reacts with:</b> Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is considered to be commercially sensitive.
Epitope	C-terminus
Positive control	WB: MCF7, Hap1, A431 and HeLa, Nuero-2a, NIH/3T3, C6, Wild-type A549 and SH-SY5Y cell lysates. IHC (FFPE): Human normal tonsil; breast carcinoma; mantle cell lymphoma; rat esophagus. ICC/IF: MCF7 cells, C6, Neuro-2a and HAP1 cells (HAP1-CCND1 knockout cells used as negative cell line). Flow Cyt (intra): MCF7, NIH/3T3 and C6 cells.
General notes	<p>This product was switched from a hybridoma to recombinant production method on 22nd January 2019.</p> <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p> <p><b>This product is FOR RESEARCH USE ONLY. For commercial use, please contact <a href="mailto:partnerships@abcam.com">partnerships@abcam.com</a>.</b></p>

#### Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid repeated freeze / thaw cycles.
Storage buffer	pH: 7.20

	Preservative: 0.1% Sodium azide
	Constituents: 1% BSA, PBS
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	SP4
<b>Isotype</b>	IgG

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab16663 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
ICC/IF	★★★★★ (5)	1/50 - 1/250.
Flow Cyt (Intra)		1/30.
WB	★★★★★ (12)	1/25 - 1/200. Detects a band of approximately 36 kDa (predicted molecular weight: 33 kDa).
IHC-P	★★★★★ (5)	1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. 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

## Target

**Function** Essential for the control of the cell cycle at the G1/S (start) transition.

**Involvement in disease** Note=A chromosomal aberration involving CCND1 may be a cause of B-lymphocytic malignancy, particularly mantle-cell lymphoma (MCL). Translocation t(11;14)(q13;q32) with immunoglobulin gene regions. Activation of CCND1 may be oncogenic by directly altering progression through the cell cycle.

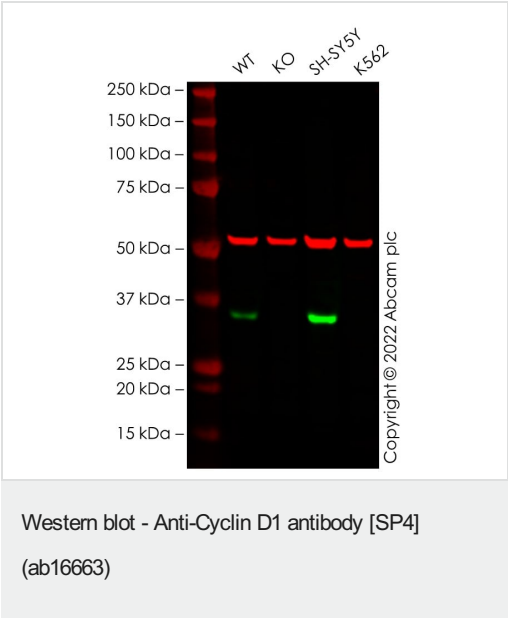
Note=A chromosomal aberration involving CCND1 may be a cause of parathyroid adenomas. Translocation t(11;11)(q13;p15) with the parathyroid hormone (PTH) enhancer.

Defects in CCND1 are a cause of multiple myeloma (MM) [MIM:254500]. MM is a malignant tumor of plasma cells usually arising in the bone marrow and characterized by diffuse involvement of the skeletal system, hyperglobulinemia, Bence-Jones proteinuria and anemia. Complications of multiple myeloma are bone pain, hypercalcemia, renal failure and spinal cord compression. The aberrant antibodies that are produced lead to impaired humoral immunity and patients have a high prevalence of infection. Amyloidosis may develop in some patients. Multiple myeloma is part of a spectrum of diseases ranging from monoclonal gammopathy of unknown significance (MGUS) to plasma cell leukemia. Note=A chromosomal aberration involving CCND1 is found in multiple myeloma. Translocation t(11;14)(q13;q32) with the IgH locus.

**Sequence similarities** Belongs to the cyclin family. Cyclin D subfamily.

<b>Post-translational modifications</b>	<p>Phosphorylation at Thr-286 by MAP kinases is required for ubiquitination and degradation following DNA damage. It probably plays an essential role for recognition by the FBXO31 component of SCF (SKP1-cullin-F-box) protein ligase complex.</p> <p>Ubiquitinated, primarily as 'Lys-48'-linked polyubiquitination. Ubiquitinated by a SCF (SKP1-CUL1-F-box protein) ubiquitin-protein ligase complex containing FBXO4 and CRYAB (By similarity). Following DNA damage it is ubiquitinated by some SCF (SKP1-cullin-F-box) protein ligase complex containing FBXO31. Ubiquitination leads to its degradation and G1 arrest. Deubiquitinated by USP2; leading to stabilize it.</p>
<b>Cellular localization</b>	Nucleus.

Images



**All lanes :** Anti-Cyclin D1 antibody [SP4] (ab16663) at 1/25 dilution

- Lane 1 :** Wild-type A549 cell lysate
- Lane 2 :** ccnd1 knockout A549 cell lysate
- Lane 3 :** SH-SY5Y cell lysate
- Lane 4 :** K562 cell lysate

Lysates/proteins at 20 µg per lane.

**Secondary**

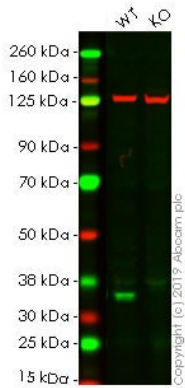
**All lanes :** Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

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

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.



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

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

**Lane 1 :** Wild-type HeLa cell lysate

**Lane 2 :** CCND1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

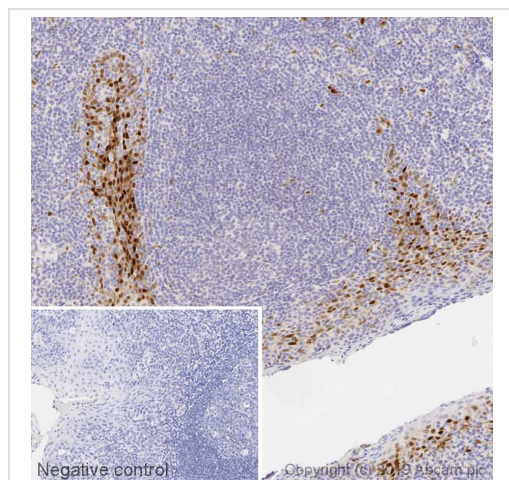
Performed under reducing conditions.

**Predicted band size:** 33 kDa

**Observed band size:** 36 kDa

**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.



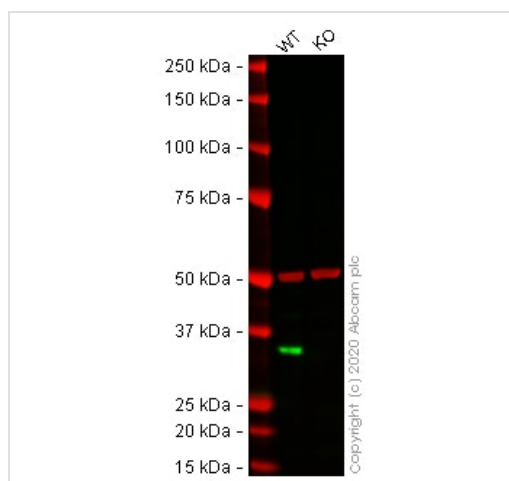
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin D1 antibody [SP4] (ab16663)

IHC image of ab16663 staining Cyclin D1 in a section of formalin-fixed paraffin-embedded normal human tonsil\* performed on a Leica BOND™ 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.



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

Anti-Cyclin D1 antibody [SP4] (ab16663) at 1/200 dilution + Wild-type HeLa cell lysate at 20 µg

Performed under reducing conditions.

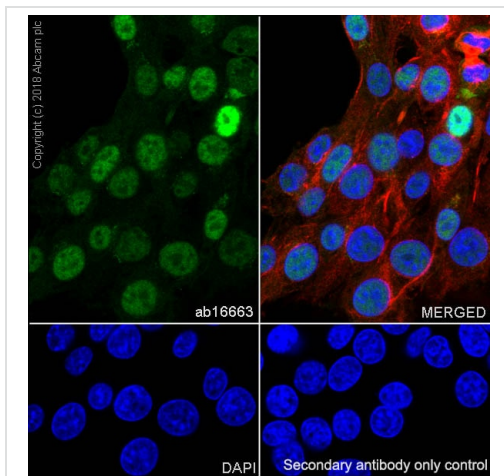
**Predicted band size:** 33 kDa

**Observed band size:** 36 kDa

**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

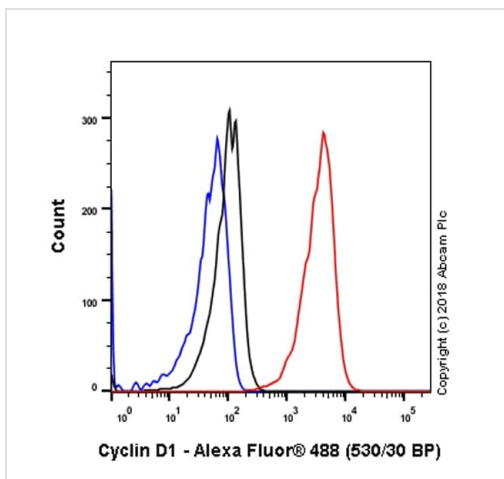
**ab261760** (knockout cell lysate **ab256864**) was used. Wild-type HeLa and CCND1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab16663** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**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.



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

Immunocytochemistry/ Immunofluorescence analysis of C6 (rat glial tumor glial cell) cells labeling Cyclin D1 with purified **ab16663** at 1/50 (5.42 µg/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.

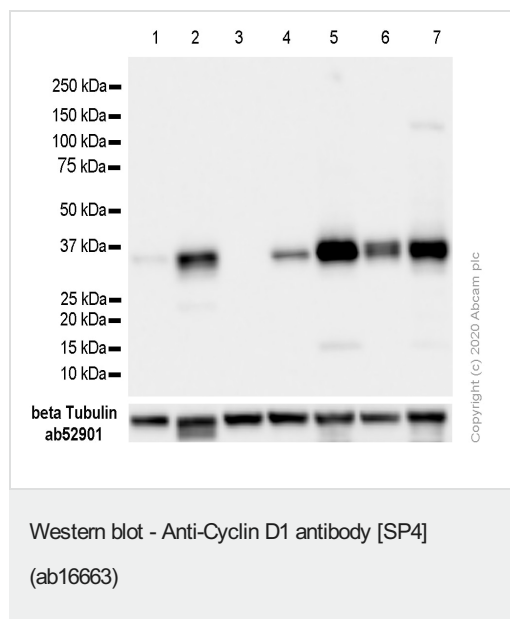
This image was generated from the hybridoma version.



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

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). Unlabeled control - Unlabelled cells (blue).

This image was generated from the hybridoma version.



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

**Lane 1 :** HeLa (Human cervix adenocarcinoma epithelial cell) cell lysate

**Lane 2 :** MCF7 (Human breast adenocarcinoma epithelial cell) cell lysate

**Lane 3 :** CCND1 KO HAP1 cell lysate

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

**Lane 5 :** Neuro-2a (Mouse neuroblastoma neuroblast) cell lysate

**Lane 6 :** NIH/3T3 (Mouse embryonic fibroblast) cell lysate

**Lane 7 :** C6 (Rat glial tumor glial cell) cell lysate

Lysates/proteins at 10 µg per lane.

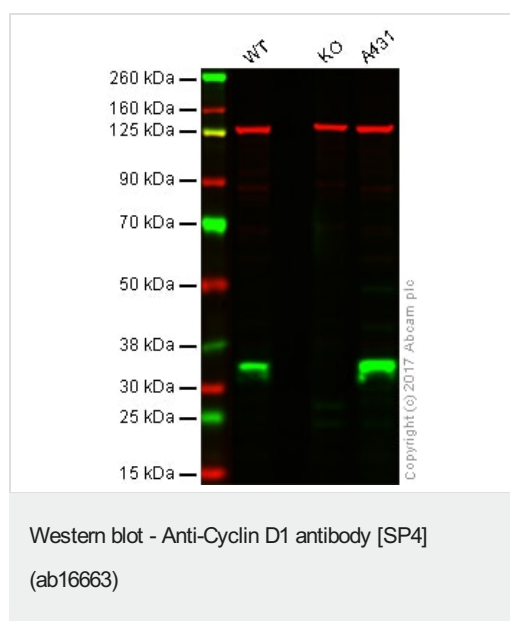
## Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

**Predicted band size:** 33 kDa

**Observed band size:** 33 kDa

**Exposure time:** 2 seconds



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

**Lane 1 :** Wild-type HAP1 whole cell lysate

**Lane 2 :** CCND1 (Cyclin D1) knockout HAP1 whole cell lysate

**Lane 3 :** A431 whole cell lysate

Lysates/proteins at 20 µg per lane.

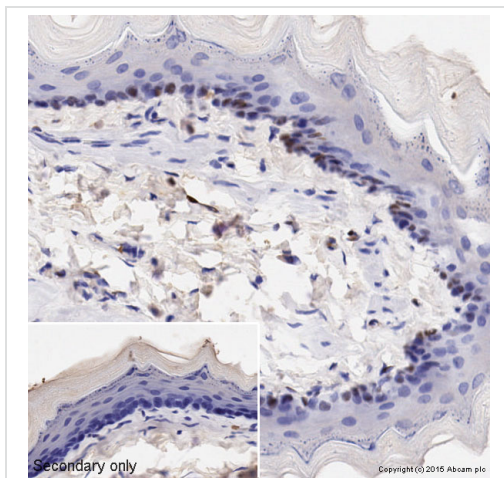
**Predicted band size:** 33 kDa

**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.

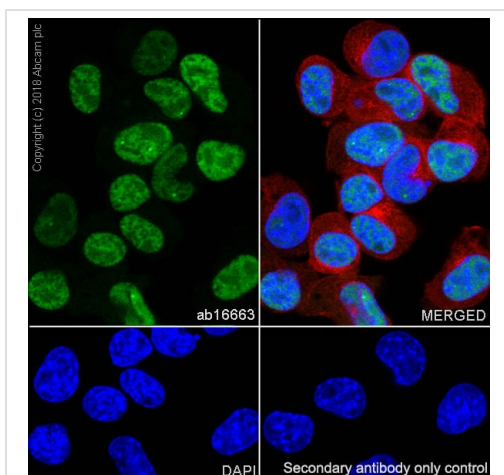


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin D1 antibody [SP4] (ab16663)

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.

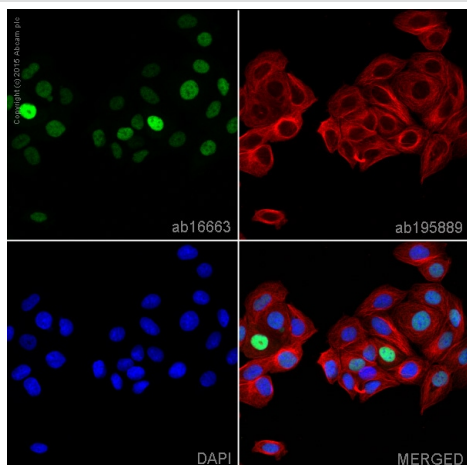
This image was generated from the hybridoma version.



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

Immunocytochemistry/ Immunofluorescence analysis of Neuro-2a (mouse neuroblastoma neuroblast) cells labeling Cyclin D1 $\mu$  with purified ab16663 at 1/50 (5.42 $\mu$ g/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  $\mu$ g/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2  $\mu$ g/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.

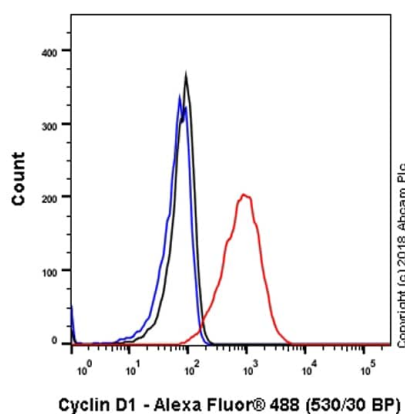


Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D1 antibody [SP4] (ab16663)

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.

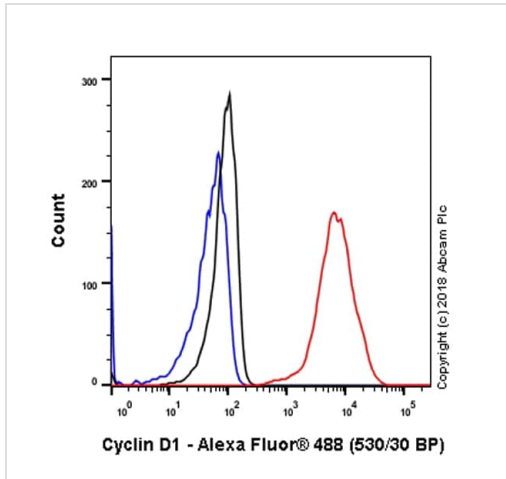
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This image was generated from the hybridoma version.



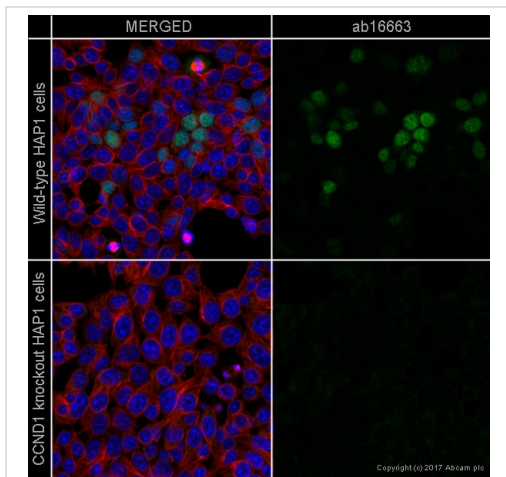
Flow Cytometry (Intracellular) - Anti-Cyclin D1 antibody [SP4] (ab16663)

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). Unlabeled control - Unlabelled cells (blue). This image was generated from the hybridoma version.



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

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). Unlabeled control - Unlabelled cells (blue). This image was generated from the hybridoma version.

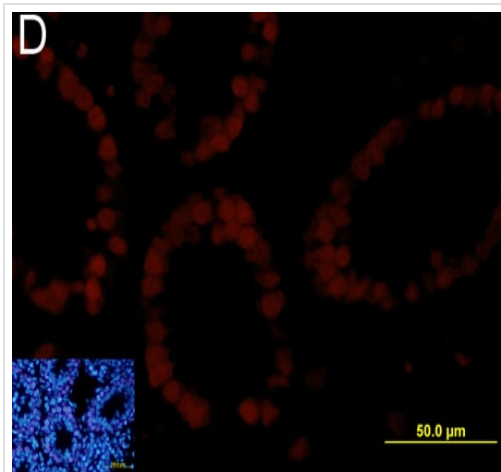


Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D1 antibody [SP4] (ab16663)

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.



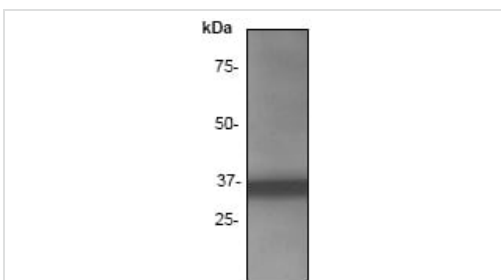
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin D1 antibody [SP4] (ab16663)

Image from Mclver 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.

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.

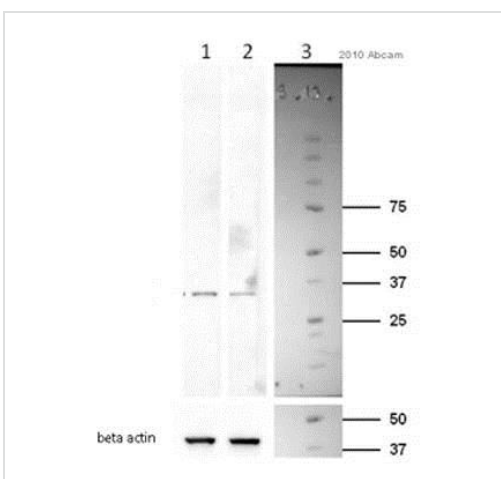


Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663)

Anti-Cyclin D1 antibody [SP4] ([ab137875](#)) at 1/5000 dilution + MCF-7 cell lysate

**Predicted band size:** 33 kDa

This image was generated from the hybridoma version.



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

Image kindly supplied by Dr Karin Birkenkamp-Demtroeder through Abreview

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

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

**All lanes :** Whole cell lysate prepared from T24 bladder cancer cells

Lysates/proteins at 25 μg per lane.

**Secondary**

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

Developed using the ECL technique.

Performed under reducing conditions.

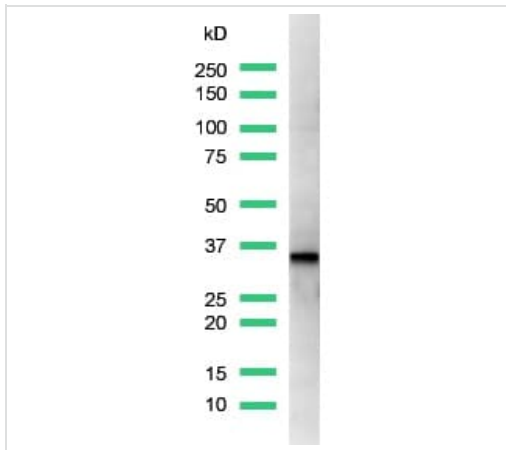
**Predicted band size:** 33 kDa

**Observed band size:** 33 kDa

**Exposure time:** 10 minutes

Gel run under denaturing conditions 4-12% gradient.

This image was generated from the hybridoma version.



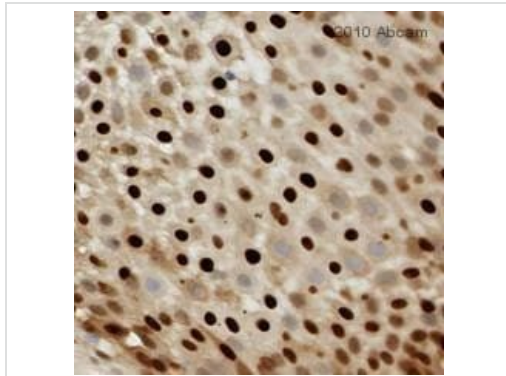
Western blot - Anti-Cyclin D1 antibody [SP4]  
(ab16663)

Anti-Cyclin D1 antibody [SP4] (ab16663) at 1/25 dilution + MCF7 cell lysate

**Predicted band size:** 33 kDa

**Observed band size:** 36 kDa

This image was generated from the hybridoma version.

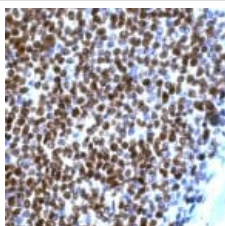


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin D1 antibody [SP4]  
(ab16663)

This image is courtesy of an Abreview submitted by Karin Birkenkamp-Demtroeder

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.



Human mantle cell lymphoma stained with ab16663.

This image was generated from the hybridoma version.

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

### Why choose a recombinant antibody?



**Research with confidence**  
Consistent and reproducible results



**Long-term and scalable supply**  
Recombinant technology



**Success from the first experiment**  
Confirmed specificity



**Ethical standards compliant**  
Animal-free production

Anti-Cyclin D1 antibody [SP4] (ab16663)

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

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- We provide support in Chinese, English, French, German, Japanese and Spanish
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