abcam

Product datasheet

Anti-Cyclin D1 antibody [SP4] ab16663

KO VALIDATED RabMAb

16 Abreviews 91 References 15 Images

Overview

Product name	Anti-Cyclin D1 antibody [SP4]
Description	Rabbit monoclonal [SP4] to Cyclin D1
Host species	Rabbit
Tested applications	**Suitable for:** ICC, ICC/IF, IHC-P, WB, IHC-P, IHC-Fr, Flow Cyt
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide corresponding to Human Cyclin D1 (C terminal).
Epitope	C-terminus
Positive control	Breast carcinomas, mantle cell lymphoma, MCF7 cell lysate IHC: Rat Esophagus (FFPE) ICC/IF: MCF7 cells, C6, Neuro-2a and HAP1 cells (HAP1-CCND1 knockout cells used as negative cell line) FC: MCF-7, NIH/3T3 and C6 cells

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: 7.50 Preservative: 0.1% Sodium azide Constituents: Tris buffered saline, 1% BSA
Purity	Immunogen affinity purified
Clonality	Monoclonal
Clone number	SP4
Isotype	IgG

Applications

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.

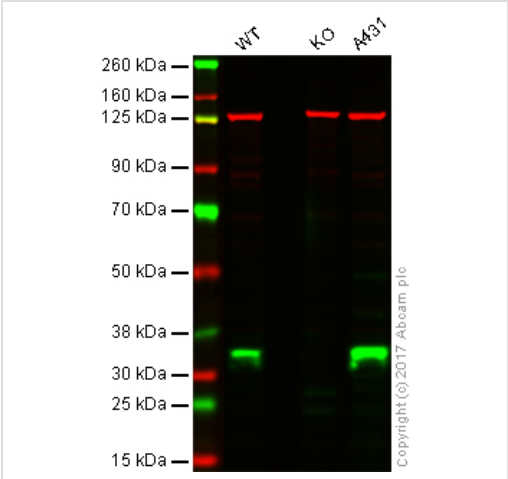
Application	Abreviews	Notes
ICC	★★★★☆	Use at an assay dependent concentration.
ICC/IF	★★★★☆	1/50 - 1/250.
IHC-P		1/100.
WB	★★★★☆	1/25 - 1/200. Detects a band of approximately 36 kDa (predicted molecular weight: 33 kDa).
IHC-P	★★★★★	1/100. Perform heat mediated antigen retrieval with citrate buffer pH 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. 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.
IHC-Fr		Use at an assay dependent concentration.
Flow Cyt		1/30.

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.
Post-translational modifications	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. 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.

Cellular localization Nucleus.

Images

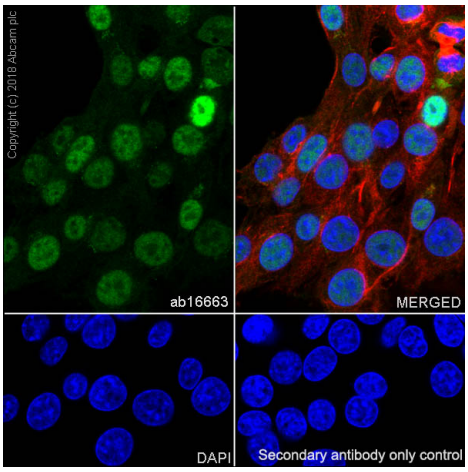


Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)
Lane 2: CCND1 (Cyclin D1) knockout HAP1 whole cell lysate (20 µg)
Lane 3: A431 whole cell lysate (20 µg)

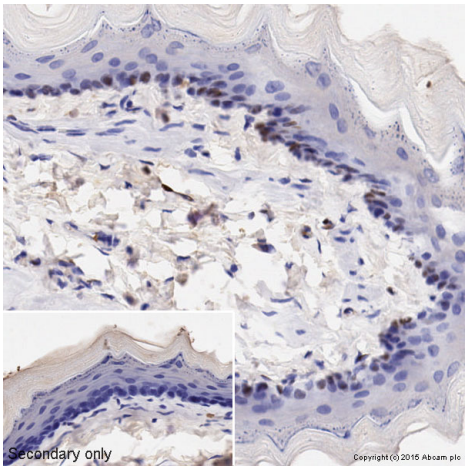
Lanes 1 - 3: Merged signal (red and green). Green - ab16663 observed at 34 kDa. Red - loading control, ab18058, observed at 130 kDa.

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.



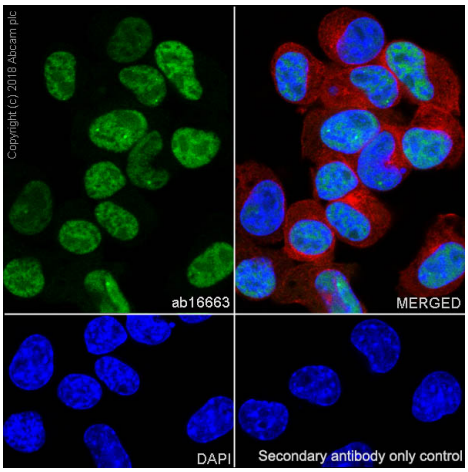
Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D1 antibody [SP4] (ab16663)

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



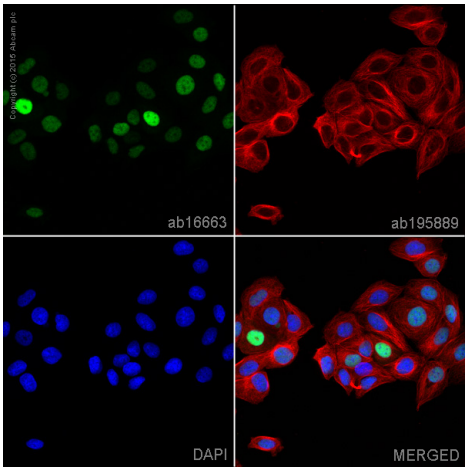
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin D1 antibody [SP4] (ab16663)

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.



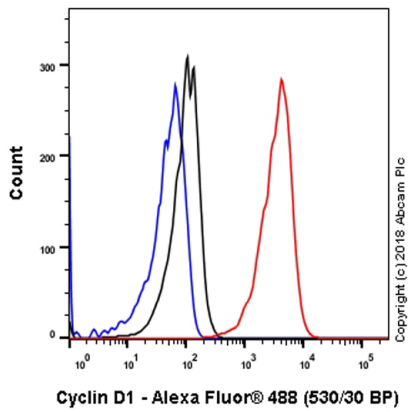
Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D1 antibody [SP4] (ab16663)

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.



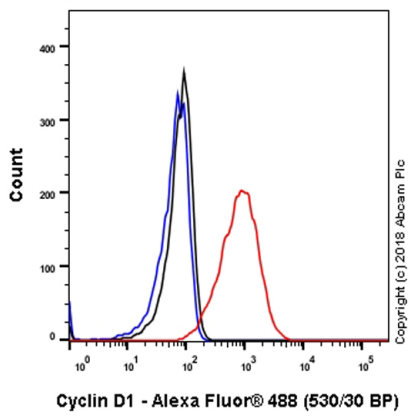
Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D1 antibody [SP4] (ab16663)

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).



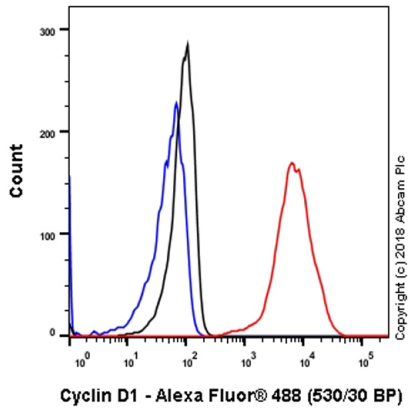
Flow Cytometry - Anti-Cyclin D1 antibody [SP4] (ab16663)

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).



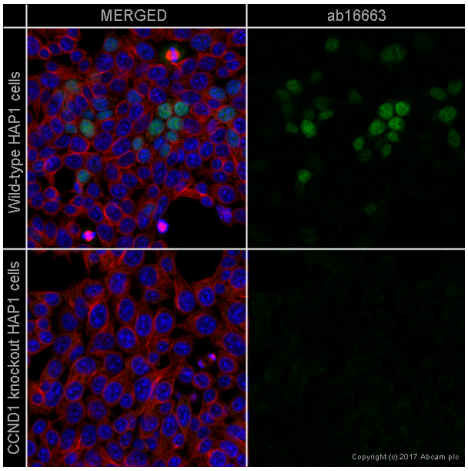
Flow Cytometry - Anti-Cyclin D1 antibody [SP4] (ab16663)

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).



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

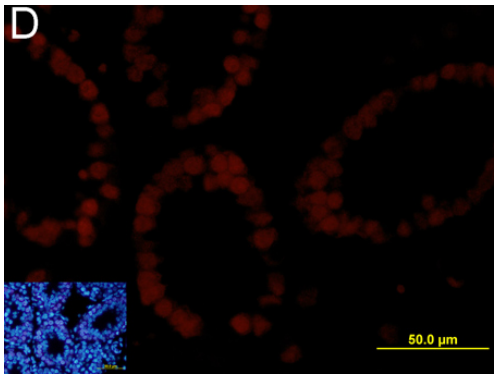
Flow cytometry analysis of NIH/3T3 (mouse embryo) labeling Cyclin D1 with purified ab16663 at 1/30 dilution (9.03 µg/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as a secondary antibody.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



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).

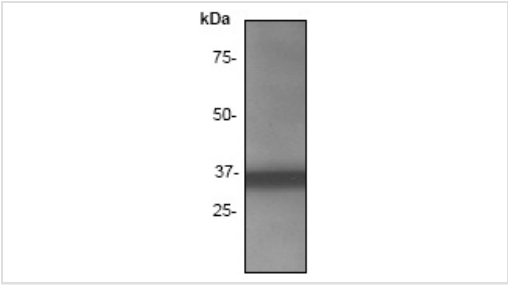


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin D1 antibody [SP4] (ab16663)

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.

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

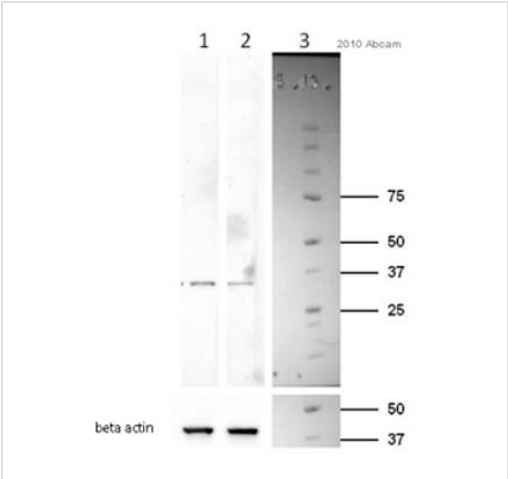
Antigen retrieval was performed via Tris-EDTA buffer. Sections were blocked with 3% BSA and incubated with primary antibody (1/50) overnight at 4°C. An AlexaFluor®594-conjugated secondary antibody was used to detect staining.



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



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

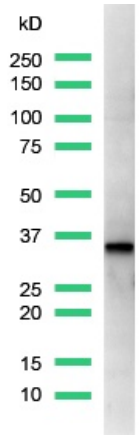
Developed using the ECL technique.

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.



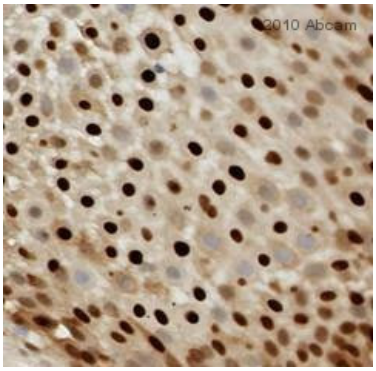
Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663)

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

Predicted band size: 33 kDa

Observed band size: 36 kDa

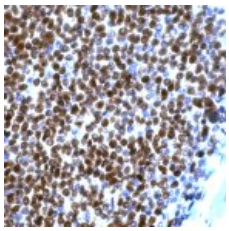
why is the actual band size different from the predicted?



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.



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

Human mantle cell lymphoma stained with ab16663.

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.com/abpromise or contact our technical team.

Terms and conditions

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