abcam

Product datasheet

Anti-Cyclin D1 antibody [SP4] ab16663

敲除验证 RabMAb

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概述

产品名称	Anti-Cyclin D1抗体[SP4]
描述	兔单克隆抗体[SP4] to Cyclin D1
经测试应用	**适用于:** ICC, ICC/IF, IHC-P, WB, IHC-P
种属反应性	**与反应:** Mouse, Human **预测可用于:** Rat
免疫原	Synthetic peptide corresponding to Human Cyclin D1 (C terminal).
表位	C-terminus
阳性对照	Breast carcinomas, mantle cell lymphoma, MCF7 cell lysate IHC: Rat Esophagus (FFPE) ICC/IF: MCF7 cells, HAP1 cells (HAP1-CCND1 knockout cells used as negative cell line)

性能

形式	Liquid
存放说明	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid repeated freeze / thaw cycles.
存储溶液	pH: 7.5 Preservative: 0.09% Sodium azide Constituents: 1% BSA, Tris buffered saline
纯度	Tissue culture supernatant
克隆	单克隆
克隆编号	SP4
同种型	IgG

应用

Our Abpromise guarantee covers the use of **ab16663** in the following tested applications.

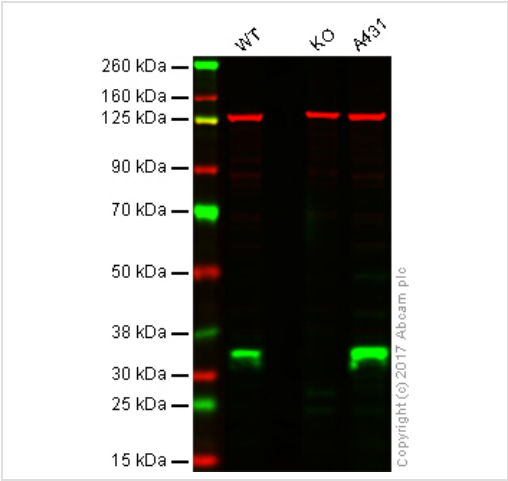
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

应用	Ab评论	说明
ICC	★★★★☆	Use at an assay dependent concentration.
ICC/IF	★★★★☆	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.

靶标

功能	Essential for the control of the cell cycle at the G1/S (start) transition.
疾病相关	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.
序列相似性	Belongs to the cyclin family. Cyclin D subfamily.
翻译后修饰	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.
细胞定位	Nucleus.

图片



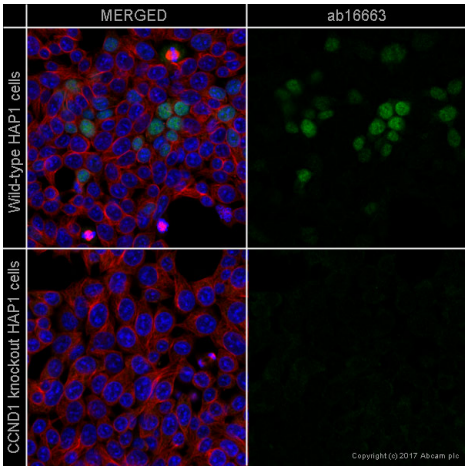
Western blot - Anti-Cyclin D1 antibody [SP4] (ab16663)

Predicted band size : 33 kDa

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.

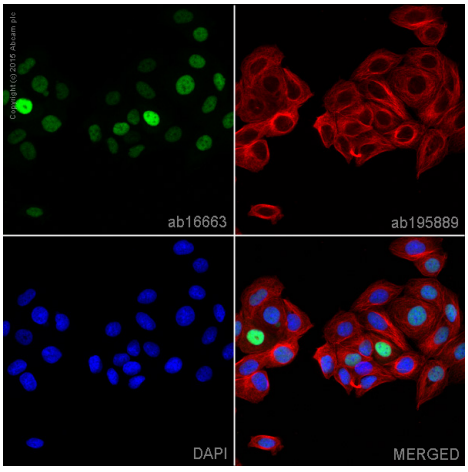
ab16663 was shown to specifically recognize CCND1 (Cyclin D1) in wild-type HAP1 cells as signal was lost at the expected MW in CCND1 (Cyclin D1) knockout cells. Wild-type and CCND1 (Cyclin D1) knockout samples were subjected to SDS-PAGE. Ab16663 and ab18058 (Mouse anti Vinculin loading control) were 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)

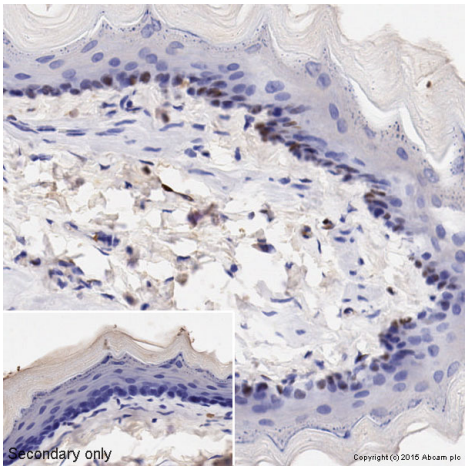
ab16663 staining Cyclin D1 in wild-type HAP1 cells (top panel) and CCND1 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The 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).



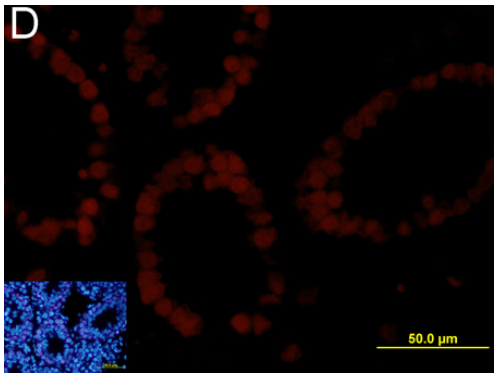
Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D1 antibody [SP4] (ab16663)

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).



Immunohistochemistry (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.

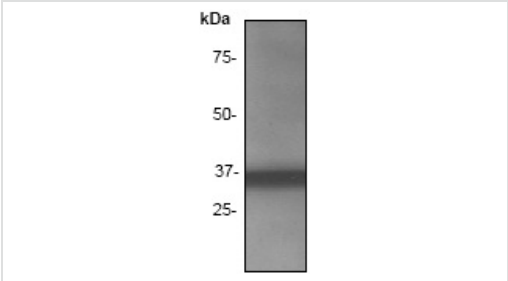


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin D1 antibody [SP4] (ab16663)

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.

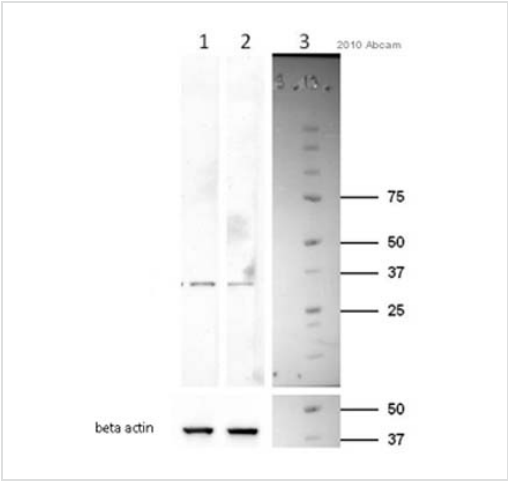
Antigen retrieval was performed via Tris-EDTA buffer. Sections were blocked with 3% BSA and incubated with primary antibody (1/50) overnight at 4°C. An AlexaFluor®594-conjugated secondary antibody was used to detect staining.



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

Lane 1 : Whole cell lysate prepared from T24 bladder cancer cells
Lane 2 : Whole cell lysate prepared from T24 bladder cancer cells

Lysates/proteins at 25 µg per lane.

Secondary
Goat anti-rabbit IgG conjugated to HRP at 1/5000 dilution
Developed using the ECL technique

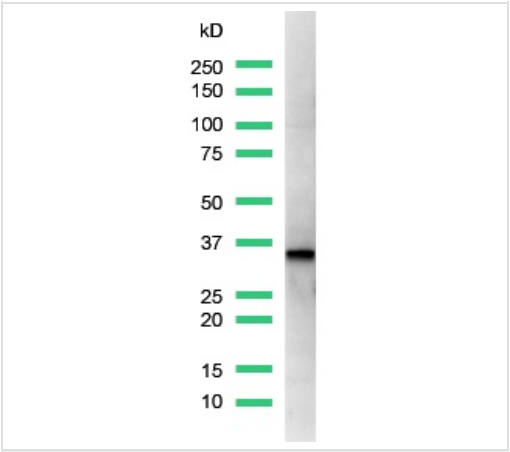
Performed under reducing conditions.

Predicted band size : 33 kDa
Observed band size : 33 kDa

Exposure time : 10 minutes

Image kindly supplied by Dr Karin Birkenkamp-Demtroeder through Abreview

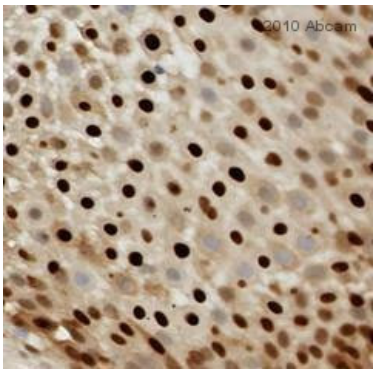
Gel run under denaturing conditions 4-12% gradient.



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

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

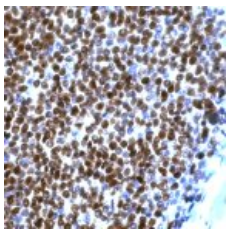
Predicted band size : 33 kDa
Observed band size : 36 kDa



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.

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