abcam

Product datasheet

Anti-Cyclin D1 antibody [SP4] - BSA and Azide free ab239794

敲除验证 重组 RabMAb

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概述

产品名称	Anti-Cyclin D1抗体[SP4] - BSA and Azide free
描述	兔单克隆抗体[SP4] to Cyclin D1 - BSA and Azide free
宿主	Rabbit
经测试应用	**适用于:** Flow Cyt (Intra), ICC/IF, WB, IHC-P
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
表位	C-terminus
阳性对照	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.
常规说明	ab239794 is the carrier-free version of **ab16663**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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 production For more information **see here**.

This product is FOR RESEARCH USE ONLY. For commercial use, please contact partnerships@abcam.com.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C. Do Not Freeze.
存储溶液	pH: 7.20 Constituent: PBS
无载体	是
纯度	Protein A purified
克隆	单克隆
克隆编号	SP4
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab239794于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
WB	★★★★★ (5)	Use at an assay dependent concentration. Detects a band of approximately 36 kDa (predicted molecular weight: 33 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval 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

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.

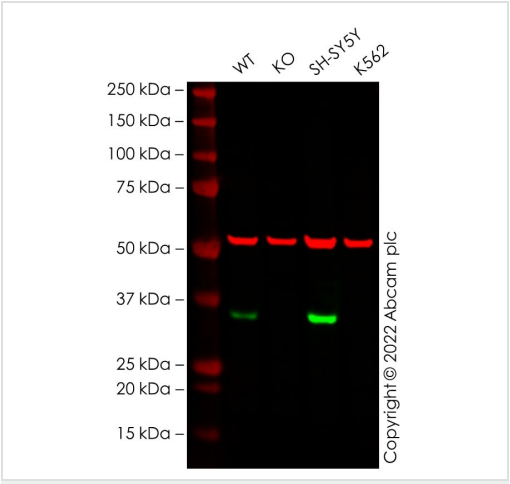
Defects in CCND1 are a cause of multiple myeloma (MM) [MIM:254500]. MM is a malignant tumor of plasma cells usually arising in the bone marrow and characterized by diffuse involvement of the skeletal system, hyperglobulinemia, Bence-Jones proteinuria and anemia. Complications of multiple myeloma are 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] - BSA and Azide free (ab239794)

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

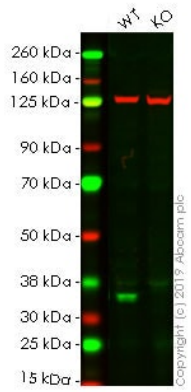
Performed under reducing conditions.

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.

This data was developed using the same antibody clone in a different buffer formulation (**ab16663**).



Western blot - Anti-Cyclin D1 antibody [SP4] - BSA and Azide free (ab239794)

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.

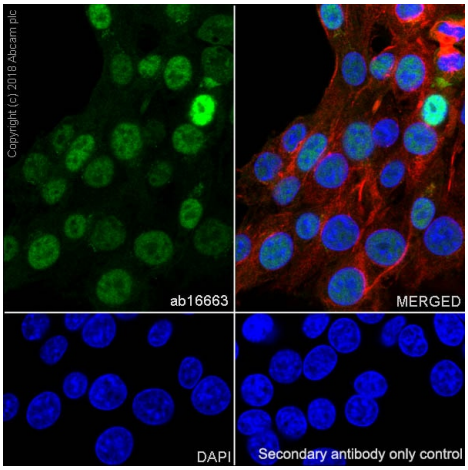
Predicted band size: 33 kDa
Observed band size: 36 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab16663**).

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 Cyclin D1 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

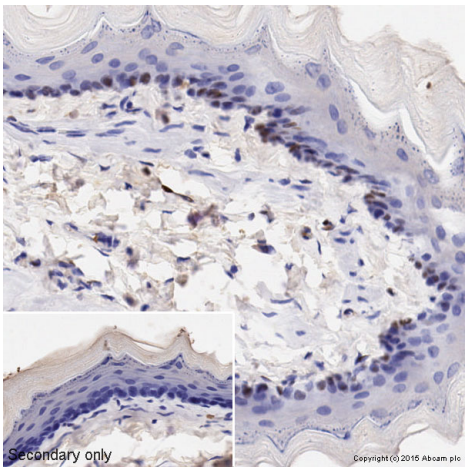
developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D1 antibody [SP4] - BSA and Azide free (ab239794)

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 data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab16663**).

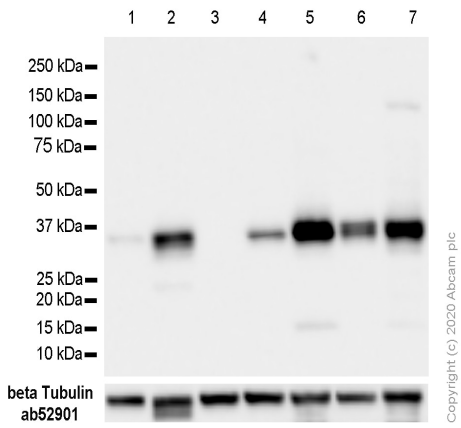


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin D1 antibody [SP4] - BSA and Azide free (ab239794)

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 data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab16663**).



Western blot - Anti-Cyclin D1 antibody [SP4] - BSA and Azide free (ab239794)

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

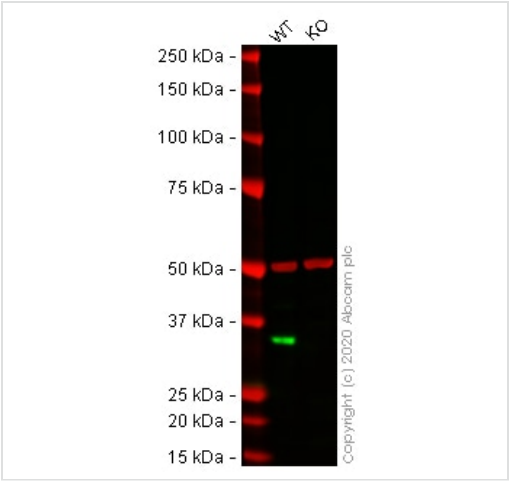
Lysates/proteins at 10 µg per lane.

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

Predicted band size: 33 kDa

Exposure time: 2 seconds

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab16663**)



Western blot - Anti-Cyclin D1 antibody [SP4] - BSA and Azide free (ab239794)

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.

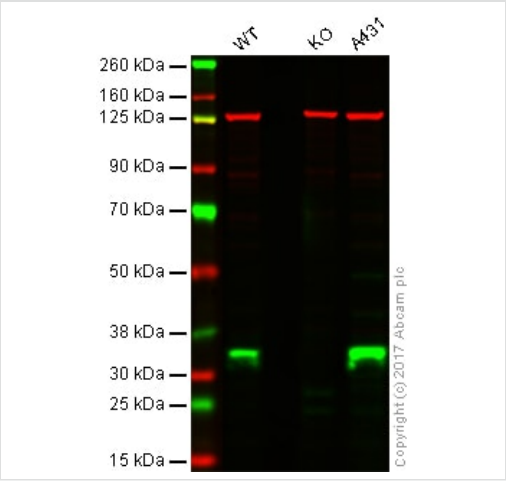
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (**ab16663**).

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.



Western blot - Anti-Cyclin D1 antibody [SP4] - BSA and Azide free (ab239794)

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

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

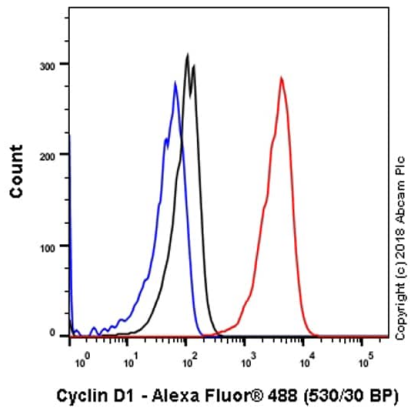
Lysates/proteins at 20 µg per lane.

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.

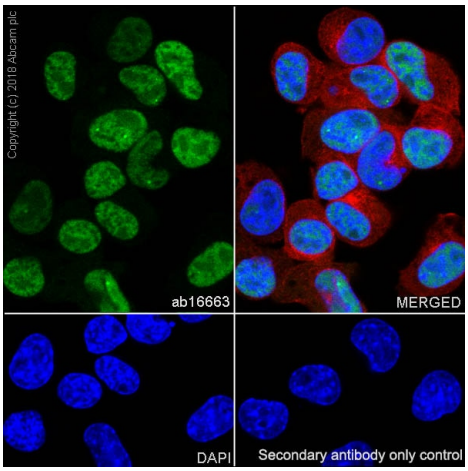
This data was developed using the same antibody clone in a different buffer formulation containing Tris buffered saline, BSA, and sodium azide (**ab16663**).



Flow Cytometry (Intracellular) - Anti-Cyclin D1 antibody [SP4] - BSA and Azide free (ab239794)

Intracellular Flow Cytometry analysis of MCF-7 (human breast carcinoma) labeling Cyclin D1 with purified **ab16663** at 1/30 dilution (9.03µg/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. Isotypecontrol - Rabbit monoclonal IgG (**ab172730**) (black). Unlableled control - Unlabelled cells (blue).

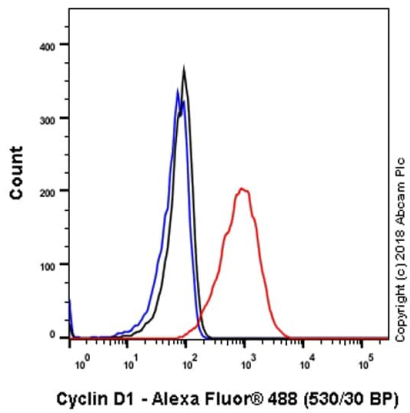
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab16663**).



Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D1 antibody [SP4] - BSA and Azide free (ab239794)

Immunocytochemistry/ Immunofluorescence analysis of Neuro-2a (mouse neuroblastoma neuroblast) 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.

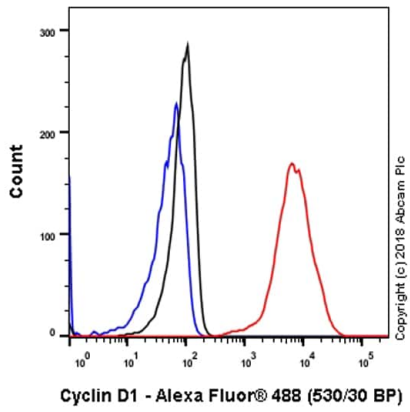
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab16663**).



Flow Cytometry (Intracellular) - Anti-Cyclin D1 antibody [SP4] - BSA and Azide free (ab239794)

Intracellular Flow Cytometry analysis of C6 (rat glioma) labeling Cyclin D1 with purified **ab16663** at 1/30 dilution (9.03µg/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. Isotypecontrol - Rabbit monoclonal IgG (**ab172730**) (black). Unlableled control - Unlabelled cells (blue).

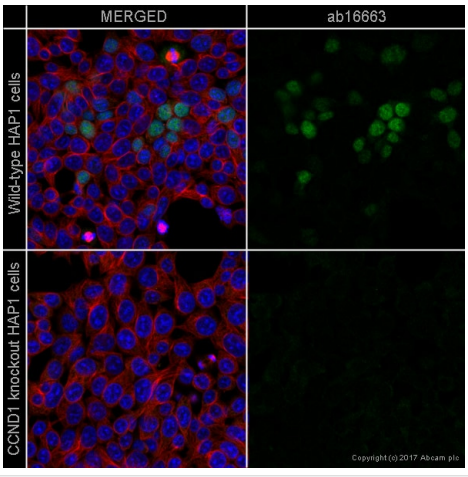
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab16663**).



Flow Cytometry (Intracellular) - Anti-Cyclin D1 antibody [SP4] - BSA and Azide free (ab239794)

Intracellular Flow Cytometry analysis of NIH/3T3 (mouse embryo) labeling Cyclin D1 with purified **ab16663** at 1/30 dilution (9.03µg/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. Isotypecontrol - Rabbit monoclonal IgG (**ab172730**) (black). Unlabeled control - Unlabelled cells (blue).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab16663**).

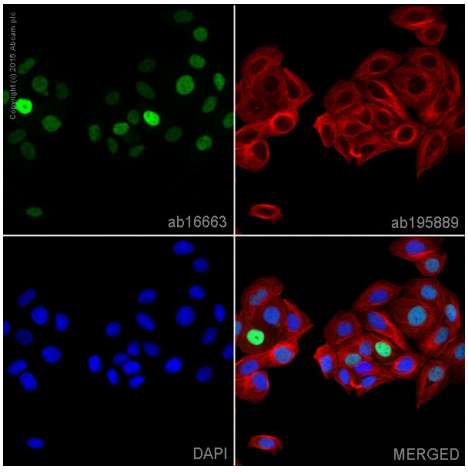


Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D1 antibody [SP4] - BSA and Azide free (ab239794)

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).

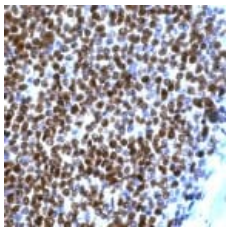
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab16663**).



Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D1 antibody [SP4] - BSA and Azide free (ab239794)

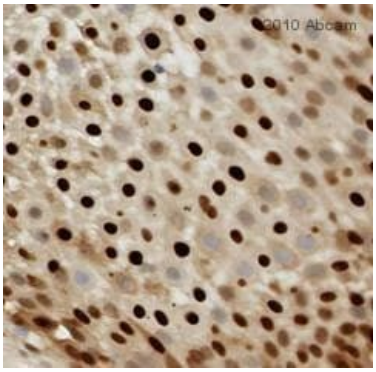
ab16663 staining Cyclin D1 in MCF7 cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab16663** at a working dilution of 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 data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab16663**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin D1 antibody [SP4] - BSA and Azide free (ab239794)

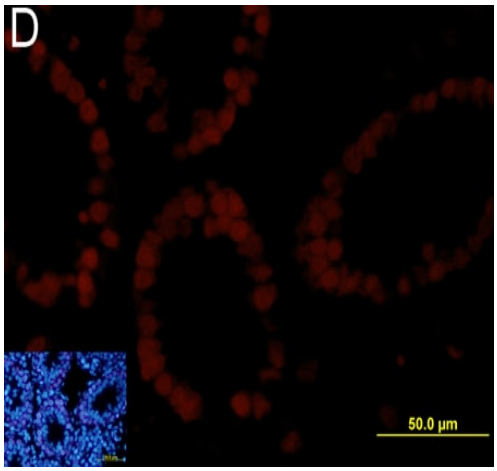
Human mantle cell lymphoma stained with **ab16663**.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab16663**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin D1 antibody [SP4] - BSA and Azide free (ab239794)

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 data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab16663**).

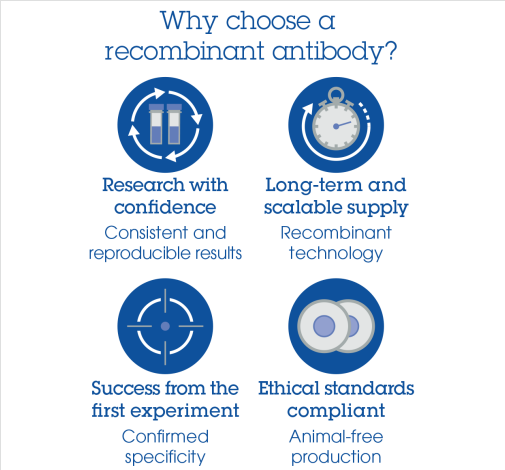


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin D1 antibody [SP4] - BSA and Azide free (ab239794)

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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab16663**).



Anti-Cyclin D1 antibody [SP4] - BSA and Azide free

(ab239794)

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

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.cn/abpromise** or contact our technical team.

Terms and conditions

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