

Product datasheet

Anti-Cyclin D1 antibody [EPR2241] - C-terminal ab134175

KO **VALIDATED** Recombinant RabMAB

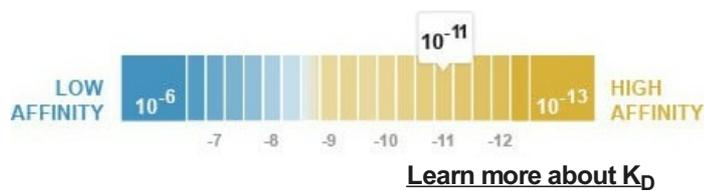
★★★★★ [15 Abreviews](#) [724 References](#) [17 Images](#)

Overview

Product name	Anti-Cyclin D1 antibody [EPR2241] - C-terminal
Description	Rabbit monoclonal [EPR2241] to Cyclin D1 - C-terminal
Host species	Rabbit
Specificity	The immunogen used for this product shares 66% homology with CCND2 (seven amino acid stretch with 100% homology). Based on internal testing in WB, this product shows a weak cross-reactivity to Cyclin D2. For IHC usage, this product shows a tissue localization specific to Cyclin D1 with no cross-reactivity to Cyclin D2.
Tested applications	Suitable for: WB, IP, ICC/IF, IHC-P
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: MCF7, LNCap, HeLa and A431 cell lysates; mouse kidney and spleen lysates; rat heart lysate; recombinant human Cyclin D 1 protein. ICC/IF: MCF7 cells treated with KN-93, Ramos cells IHC-P: Human esophagus, rat esophagus and human mantle cell lymphoma tissue. IP: A431 cell lysate.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none">- High batch-to-batch consistency and reproducibility- Improved sensitivity and specificity- Long-term security of supply- Animal-free production <p>For more information see here.</p> <p>Our RabMAB[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAB[®] patents.</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.

Dissociation constant (K_D)K_D = 2.40 x 10⁻¹¹ M**Storage buffer**

pH: 7.2
 Preservative: 0.01% Sodium azide
 Constituents: 40% Glycerol, 0.05% BSA, PBS

Purity

Protein A purified

Clonality

Monoclonal

Clone number

EPR2241

Isotype

IgG

Applications**The Abpromise guarantee**Our **Abpromise guarantee** covers the use of ab134175 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
WB	★★★★★ (12)	1/10000 - 1/50000. Predicted molecular weight: 34 kDa. Compared to the working concentration with cyclin D1 recombinant protein, a 50 fold increase is required with cyclin D2 recombinant protein in western blot testing.
IP		1/30.
ICC/IF	★★★★★ (2)	1/50.
IHC-P		1/100 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols .

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

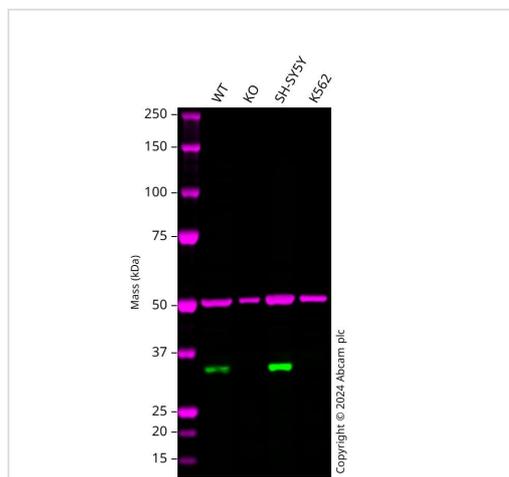
Phosphorylation at Thr-286 by MAP kinases is required for ubiquitination and degradation following DNA damage. It probably plays an essential role for recognition by the FBXO31 component of SCF (SKP1-cullin-F-box) protein ligase complex.

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 [EPR2241] - C-terminal (ab134175)

All lanes : Anti-Cyclin D1 antibody [EPR2241] - C-terminal (ab134175) at 1/10000 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.

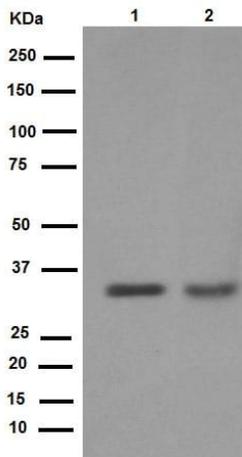
Performed under reducing conditions.

Predicted band size: 34 kDa

Observed band size: 34 kDa

Western blot: Anti-CCND1 antibody [EPR2241] (ab134175) staining at 1/10000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab134175 was shown to bind specifically to CCND1. A band was observed at 34 kDa in wild-type A549 cell lysates with no signal observed at this size in CCND1 knockout cell line. 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 [EPR2241] - C-terminal (ab134175)

All lanes : Anti-Cyclin D1 antibody [EPR2241] - C-terminal (ab134175) at 1/100000 dilution (purified)

Lane 1 : MCF7 (Human breast adenocarcinoma cell line) cell lysate

Lane 2 : LNCap (Human prostate cancer cell line) cell lysate

Lysates/proteins at 20 µg per lane.

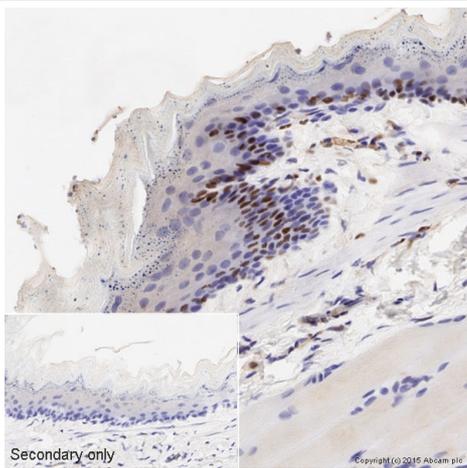
Secondary

All lanes : HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 34 kDa

Observed band size: 34 kDa

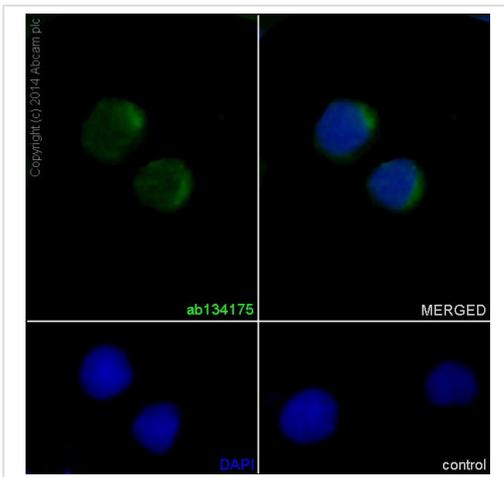
Blocking/Dilution buffer: 5% NFDm/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin D1 antibody [EPR2241] - C-terminal (ab134175)

IHC image of ab134175 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 ab134175, 5µg/ml working concentration, 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 hematoxylin and mounted with DPX. No primary antibody was used in the secondary only control (shown on the inset).

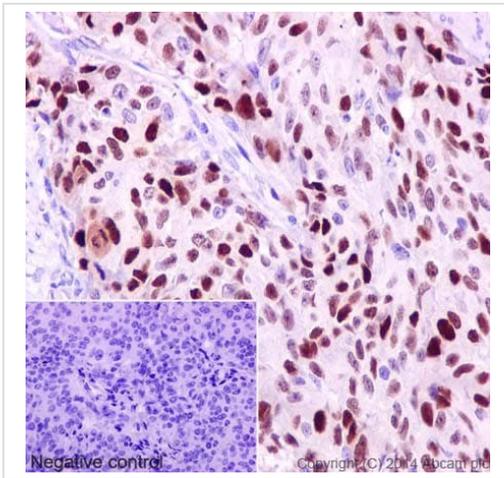
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D1 antibody [EPR2241] - C-terminal (ab134175)

Immunofluorescent staining of Ramos (Human Burkitt's lymphoma cell line) cells (fixed in 4% PFA, permeabilized with 0.1% Triton X 100) using purified ab134175 at a dilution of 1/50. An Alexa Fluor[®] 488 goat anti-rabbit antibody was used as the secondary at a dilution of 1/500 and the cells were counterstained with DAPI.

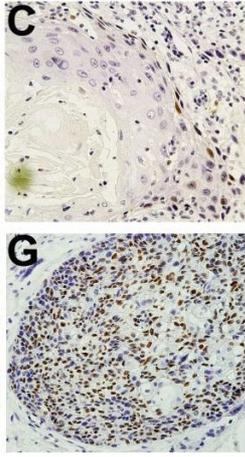
The negative control is shown in the bottom right hand panel - for the negative control, Alex Fluor[®] 594 goat anti-mouse was used at a dilution of 1/500.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin D1 antibody [EPR2241] - C-terminal (ab134175)

Immunohistochemical staining of paraffin embedded human endometrial adenocarcinoma with purified ab134175 at a dilution of 1/100. An HRP goat anti-rabbit (**ab97051**) was used as the secondary antibody at a dilution of 1/500 and the sample was counterstained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0.

PBS was used instead of the primary antibody as the negative control (inset).



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

[EPR2241] - C-terminal (ab134175)

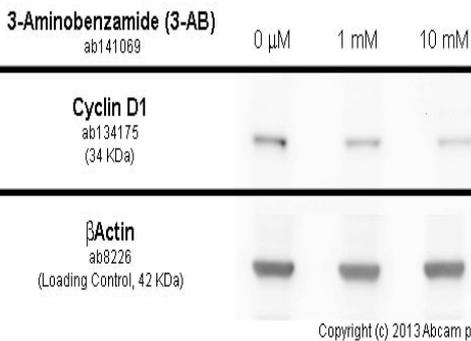
Feng et al PLoS One. 2011;6(10):e26399. doi: 10.1371/journal.pone.0026399. Epub 2011 Oct 31. Fig 1. Reproduced under the Creative Commons license <http://creativecommons.org/licenses/by/4.0/>

Immunohistochemistry was performed on human head and neck squamous cell carcinoma tissue using a rabbit monoclonal antibody against the CCND1 protein ab134175 on 3- μ m slides using 224 paraffin sections via the standard SP method.

Panel C: An example of low Cyclin D1 expression.

Panel G: An example of high Cyclin D1 expression.

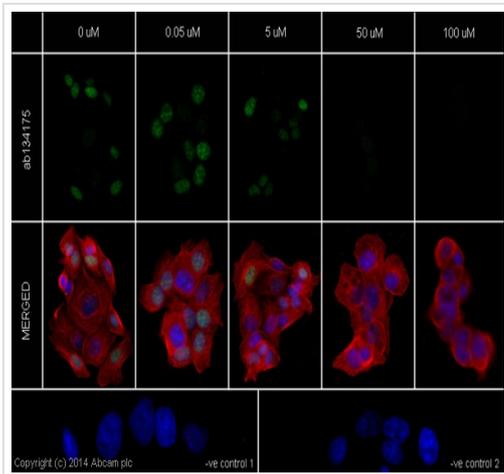
For full image please see paper.



Western blot - Anti-Cyclin D1 antibody [EPR2241] - C-terminal (ab134175)

MG-63 cells were incubated at 37°C for 24h with vehicle control (0 μ M) and different concentrations of 3-aminobenzamide (3-AB) (**ab141069**). Decreased expression of cyclin D1 (unpurified ab134175) in MG-63 cells correlates with an increase in 3-aminobenzamide (3-AB) concentration, as described in literature.

Whole cell lysates were prepared with RIPA buffer (containing protease inhibitors and sodium orthovanadate), 10 μ g of each were loaded on the gel and the WB was run under reducing conditions. After transfer the membrane was blocked for an hour using 5% BSA before being incubated with unpurified ab134175 at 1/500 dilution and **ab8226** at 1 μ g/ml overnight at 4°C. Antibody binding was detected using an **anti-rabbit HRP** secondary antibody (**ab97051**) at 1/10000 dilution and visualised using ECL development solution.

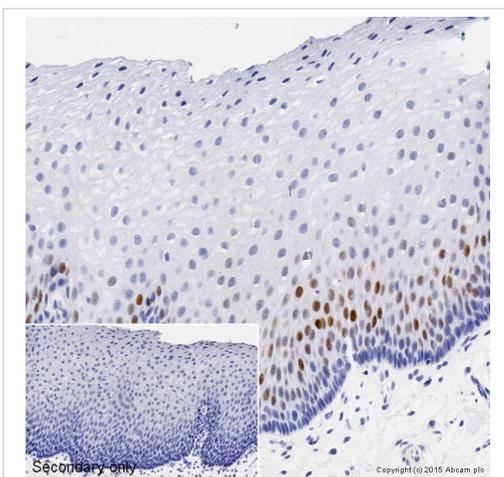


Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D1 antibody [EPR2241] - C-terminal (ab134175)

Unpurified ab134175 staining Cyclin D1 in MCF7 (Human breast adenocarcinoma cell line) cells treated with KN-93 (**ab120980**).

The cells were fixed with 100% methanol (5min) 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 ab134175 at 10μg/ml and **ab7291** at 1μg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with an Goat **anti-Rabbit Alexa 488** secondary (**ab150081**) at 2 μg/ml (shown in green) and Goat **anti-Mouse Alexa 594** secondary (**ab150120**) at 2 μg/ml (shown in pseudo color red). Nuclear DNA was labeled in blue with DAPI.

Negative controls: 1– Rabbit primary and anti-mouse secondary antibody; 2 – Mouse primary antibody and anti-rabbit secondary antibody. Controls 1 and 2 indicate that there is no unspecific reaction between primary and secondary antibodies used.

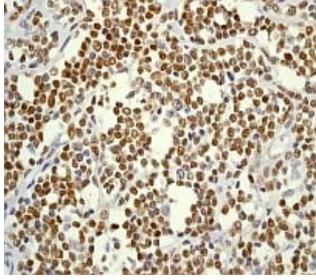


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin D1 antibody [EPR2241] - C-terminal (ab134175)

IHC image of ab134175 staining Cyclin D1 in human 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 ab134175, 5μg/ml working concentration, 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 hematoxylin 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.

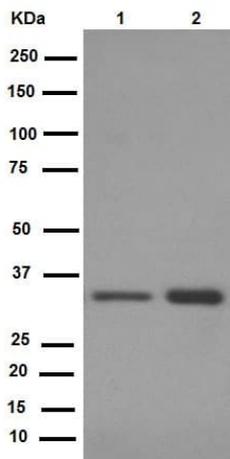
*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin D1 antibody [EPR2241] - C-terminal (ab134175)

Immunohistochemical analysis of paraffin-embedded human mantle cell lymphoma tissue, labeling Cyclin D1 with unpurified ab134175 at 1/100 dilution.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Western blot - Anti-Cyclin D1 antibody [EPR2241] - C-terminal (ab134175)

All lanes : Anti-Cyclin D1 antibody [EPR2241] - C-terminal (ab134175) at 1/20000 dilution (purified)

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

Lane 2 : A431 (Human epidermoid carcinoma cell line) cell lysate

Lysates/proteins at 20 µg per lane.

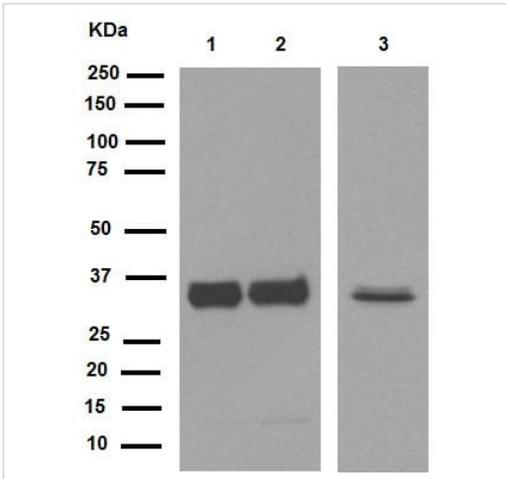
Secondary

All lanes : HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 34 kDa

Observed band size: 34 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Cyclin D1 antibody [EPR2241] - C-terminal (ab134175)

All lanes : Anti-Cyclin D1 antibody [EPR2241] - C-terminal (ab134175) at 1/10000 dilution (purified)

Lane 1 : Mouse kidney

Lane 2 : Mouse spleen

Lane 3 : Rat heart

Lysates/proteins at 20 µg per lane.

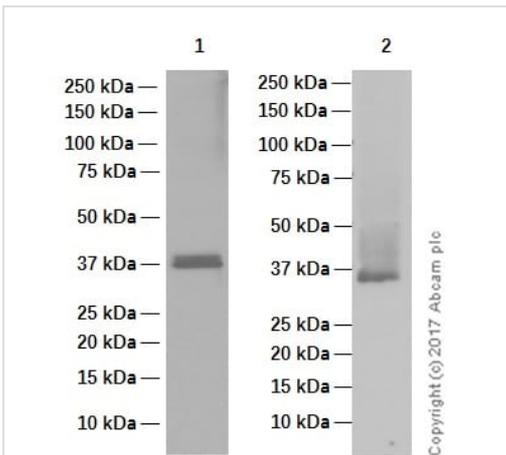
Secondary

All lanes : HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 34 kDa

Observed band size: 34 kDa

Blocking/Dilution buffer: 5% NFDm/TBST.



Western blot - Anti-Cyclin D1 antibody [EPR2241] - C-terminal (ab134175)

Lane 1 : Anti-Cyclin D1 antibody [EPR2241] - C-terminal (ab134175) at 0.04 µg/ml

Lane 2 : Anti-Cyclin D1 antibody [EPR2241] - C-terminal (ab134175) at 2 µg/ml

Lane 1 : His-Tagged full-length human Cyclin D1 (aa 1 to 295) recombinant protein

Lane 2 : His-Tagged full-length human Cyclin D2 (aa 1 to 289) recombinant protein

Lysates/proteins at 0.015 µg per lane.

Secondary

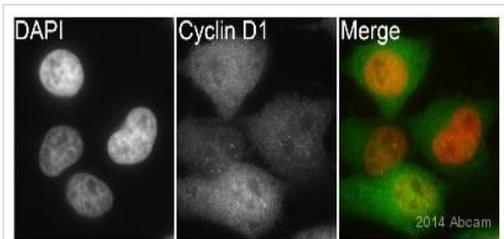
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 34 kDa

Observed band size: 37 kDa

Exposure time: 3 minutes

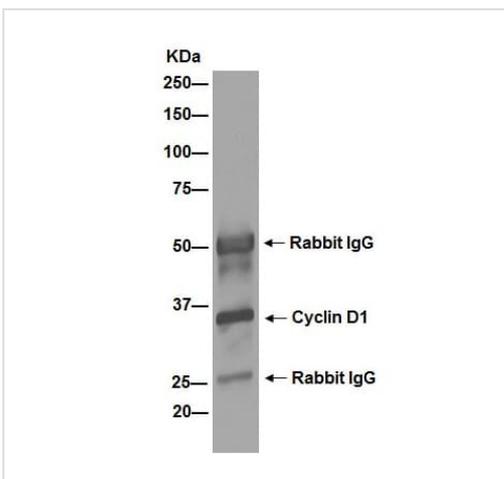
Blocking and dilution buffer: 5% NFDm/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-Cyclin D1 antibody [EPR2241] - C-terminal (ab134175)

This image is courtesy of an Abreview submitted by Kirk Mcmanus.

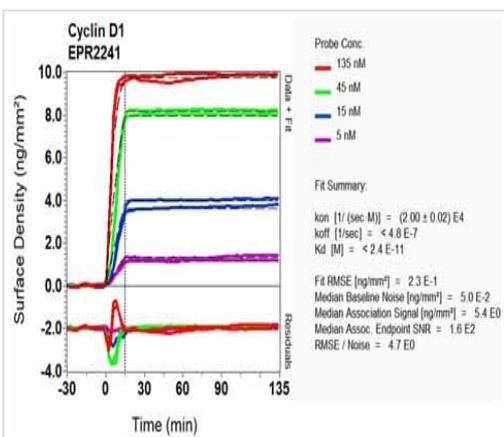
Immunocytochemical analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells, labeling Cyclin D1 with ab134175 at a dilution of 1/200. Cells were paraformaldehyde fixed and permeabilized with 0.5% Triton X-100 in PBS. Incubation with the primary antibody was for 1 hour at 22°C. Cells were counterstained with DAPI following immunostaining.



Immunoprecipitation - Anti-Cyclin D1 antibody [EPR2241] - C-terminal (ab134175)

ab134175 (purified) at 1/30 immunoprecipitating cyclin D1 in A431 (Human epidermoid carcinoma cell line) cells. For western blotting, an HRP-conjugated goat anti-rabbit IgG, was used as the secondary antibody (1/1000).

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



OI-RD Scanning - Anti-Cyclin D1 antibody [EPR2241] - C-terminal (ab134175)

Equilibrium disassociation constant (K_D)

Learn more about K_D

[Click here to learn more about \$K_D\$](#)

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 [EPR2241] - C-terminal
(ab134175)

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