abcam

ab133266

Anti-Cyclin E1 antibody [EPR194]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR194
Purification technique	Affinity purification Protein A
Concentration	1.513 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human
Dilution info	1/1000 - 1/10000
Notes	-

IHC-P

Not recommended

Species	Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/1000
Notes	**For unpurified use at 1:500.**

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/150
Notes	-

Target data

See full target information CCNE1

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

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C

Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Species reactivity
Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

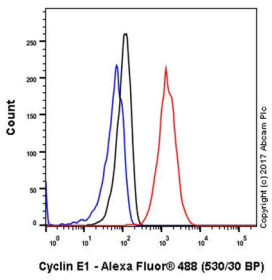
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

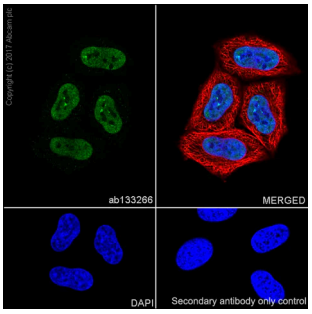
12 product images



Flow Cytometry (Intracellular) - Anti-Cyclin E1 antibody [EPR194] (ab133266)

Cyclin E1 Flow Cytometry (Intracellular) staining using rabbit Anti-Cyclin E1 antibody

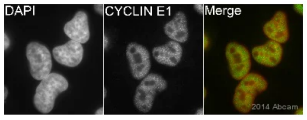
Intracellular Flow Cytometry analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling Cyclin E1 with purified ab133266 at 1/150 dilution (10 µg/ml) (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunocytochemistry/ Immunofluorescence - Anti-Cyclin E1 antibody [EPR194] (ab133266)

Cyclin E1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Cyclin E1 antibody

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Cyclin E1 with Purified ab133266 at 1:1000 dilution (1.6µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). ab150077 Goat anti rabbit IgG(Alexa Fluor® 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

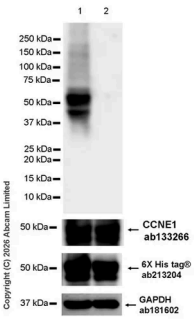


This image is courtesy of a customer review submitted by Kirk McManus

Immunocytochemistry/ Immunofluorescence - Anti-Cyclin E1 antibody [EPR194] (ab133266)

Cyclin E1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Cyclin E1 antibody

Unpurified ab133266 staining Cyclin E1 in HeLa cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde and permeabilized with 0.5% Triton X-100 in PBS. Samples were incubated with primary antibody (1/500 in PBS) for 1 hour at 22°C. ab150081, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG polyclonal (1/200) was used as the secondary antibody. Counterstained with DAPI.



Western blot - Anti-Cyclin E1 antibody [EPR194] (ab133266)

Cyclin E1 Western blot staining using rabbit Anti-Cyclin E1 antibody

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

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) (1:200000) (36KDa); Anti-6X His tag antibody [EPR20547]-CHIP Grade (ab213204) (1:5000)(35-50KDa).

All lanes:
Western blot - Anti-Cyclin E1(Phospho T77) antibody [EPR28889-10] (ab327426) at 1/5000 dilution

Lane 1:
293T cells transfected with a human wild-type CCNE1 expression vector containing a His-tag, whole cell lysate at 20 µg

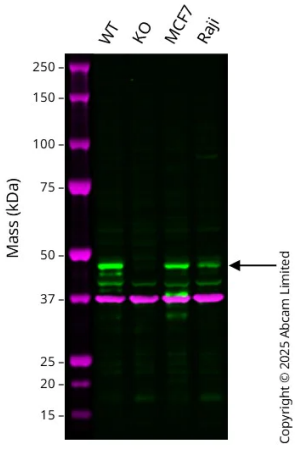
Lane 2:
293T cells transfected with a human CCNE1 (T77A) expression vector containing a His-tag, whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/200000 dilution

Observed band size: 35-50 kDa, 36 kDa

Exposure time: 15s



Western blot - Anti-Cyclin E1 antibody [EPR194] (ab133266)

Cyclin E1 Western blot staining using rabbit Anti-Cyclin E1 antibody

Western blot: Rabbit Monoclonal[EPR194] to Cyclin E1 ab133266 staining at 1/1000 dilution, shown in green; Mouse anti GAPDH ab8245 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 47 kDa in Wild-type A549 cell lysates with no signal observed at this size in CCNE1 knockout A549 cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3pc 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 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-Cyclin E1 antibody [EPR194] (ab133266) at 1/1000 dilution

Lane 1:
Wild-type A549 at 20 µg

Lane 2:
Western blot - Human CCNE1 knockout A549 cell line (ab286681) at 20 µg

Lane 3:
MCF7 at 20 µg

Lane 4:
Raji at 20 µg

Secondary

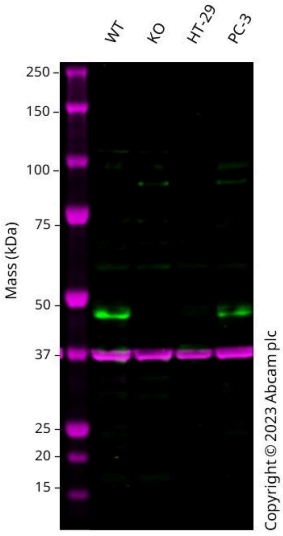
All lanes:

Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 47 kDa

Observed band size: 47 kDa, 36 kDa



Western blot - Anti-Cyclin E1 antibody [EPR194] (ab133266)

Western blot: Anti-CCNE1 antibody [EPR194] (ab133266) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab133266 was shown to bind specifically to CCNE1. A band was observed at 47 kDa in wild-type MCF7 cell lysates with no signal observed at this size in CCNE1 knockout cell line ab286303 (knockout cell lysate ab300211). To generate this image, wild-type and CCNE1 knockout MCF7 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.
All lanes:
Western blot - Anti-Cyclin E1 antibody [EPR194] (ab133266) at 1/1000 dilution

Lane 1:
Wild-type MCF7 cell lysate at 20 µg

Lane 2:
CCNE1 knockout MCF7 cell lysate at 20 µg

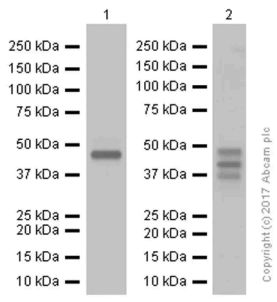
Lane 3:
HT-29 cell lysate at 20 µg

Lane 4:
PC-3 cell lysate at 20 µg

Developed using the ECL technique.

Performed under reducing conditions.

Observed band size: 47 kDa



Western blot - Anti-Cyclin E1 antibody [EPR194] (ab133266)

Blocking and diluting buffer : 5% NFDM/TBST
All lanes:
Western blot - Anti-Cyclin E1 antibody [EPR194] (ab133266) at 1/10000 dilution

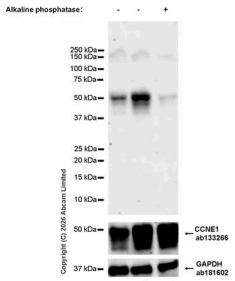
Lane 1:
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates at 15 µg

Lane 2:
JAR (Human placenta choriocarcinoma epithelial cell) whole cell lysates at 15 µg

Secondary

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

Predicted band size: 47 kDa



Western blot - Anti-Cyclin E1 antibody [EPR194] (ab133266)

Cyclin E1 Western blot staining using rabbit Anti-Cyclin E1 antibody

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

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) (1:200000) (36KDa).

All lanes:
Western blot - Anti-Cyclin E1(Phospho T77) antibody [EPR28889-10] (ab327426) at 1/1000 dilution

Lane 1:
Untreated A549 (human lung carcinoma epithelial cell) whole cell lysate (untreated membrane) at 50 µg

Lane 2:
A549 treated with 2.5mM Thymidine for 16h whole cell lysate (untreated membrane) at 50 µg

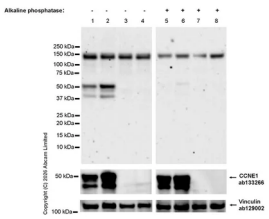
Lane 3:
A549 treated with 2.5mM Thymidine for 16h whole cell lysate (alkaline phosphatase treated membrane) at 50 µg

Secondary

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

Observed band size: 35-50 kDa, 36 kDa

Exposure time: 48s



Western blot - Anti-Cyclin E1 antibody [EPR194] (ab133266)

Cyclin E1 Western blot staining using rabbit Anti-Cyclin E1 antibody

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

In Western blot, ab327426 was shown to bind specifically to CCNE1. Target of interest was observed at 35-50 kD in wild-type HCT116 cell lysates (lanes 1-2) with no signal observed at this size in the CCNE1 knockout cell line (lanes 3-4) (lanes 3-4, knockout cell line ab286494).

The identity of the higher MW band at approximately 150 kDa is unknown.

In Western blot, Anti-Vinculin antibody [EPR8185] (ab129002) (1:10000) (124KDa).

All lanes:
Western blot - Anti-Cyclin E1(Phospho T77) antibody [EPR28889-10] (ab327426) at 1/1000 dilution

Lane 1:
Untreated HCT 116 (human colorectal carcinoma epithelial cell) whole cell lysate (untreated membrane) at 50 µg

Lane 2:
HCT 116 treated with 2.5mM Thymidine for 16h whole cell lysate (untreated membrane) at 50 µg

Lane 3:
CCNE1 knockout HCT116 whole cell lysate (untreated membrane) at 50 µg

Lane 4:
CCNE1 knockout HCT116 treated with 2.5mM Thymidine for 16h) whole cell lysate (untreated membrane) at 50 µg

Lane 5:
Untreated HCT 116 whole cell lysate (alkaline phosphatase treated membrane) at 50 µg

Lane 6:
HCT 116 treated with 2.5mM Thymidine for 16h whole cell lysate (alkaline phosphatase treated membrane) at 50 µg

Lane 7:
CCNE1 knockout HCT116 whole cell lysate (alkaline phosphatase treated membrane) at 50 µg

Lane 8:
CCNE1 knockout HCT116 treated with 2.5mM Thymidine for 16h whole cell lysate (alkaline phosphatase treated membrane) at 50 µg

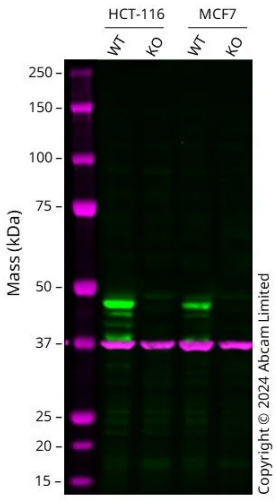
Secondary

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

Performed under reducing conditions.

Observed band size: 35-50 kDa, 124 kDa

Exposure time: 180s



Western blot - Anti-Cyclin E1 antibody [EPR194] (ab133266)

Cyclin E1 Western blot staining using rabbit Anti-Cyclin E1 antibody

Western blot: Anti-CCNE1 antibody [EPR194] (ab133266) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab133266 was shown to bind specifically to CCNE1. A band was observed at 45 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in CCNE1 knockout cell line. To generate this image, wild-type and CCNE1 knockout HCT 116 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.
All lanes:
Western blot - Anti-Cyclin E1 antibody [EPR194] (ab133266) at 1/1000 dilution

Lane 1:
Wild-type HCT 116 cell lysate at 20 µg

Lane 2:
CCNE1 knockout HCT 116 cell lysate at 20 µg

Lane 3:
Wild-type MCF7 ab288560 cell lysate at 20 µg

Lane 4:
CCNE1 knockout MCF7 ab286303 cell lysate at 20 µg

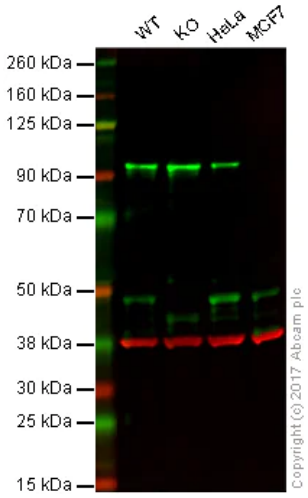
Secondary

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: 47 kDa

Observed band size: 45 kDa



Western blot - Anti-Cyclin E1 antibody [EPR194] (ab133266)

Cyclin E1 Western blot staining using rabbit Anti-Cyclin E1 antibody

Lanes 1 - 4: Merged signal (red and green). Green - ab133266 observed at 47 kDa. Red - loading control, ab9484, observed at 37 kDa.

Unpurified ab133266 was shown to recognize Cyclin E1 in wild-type cells as signal was lost at the expected MW in Cyclin E1 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and Cyclin E1 knockout samples were subjected to SDS-PAGE. ab133266 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 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 1/20000 dilution for 1 hour at room temperature before imaging.

Lane 1:
Western blot - Anti-Cyclin E1 antibody [EPR194] (ab133266) at 1/1000 dilution

Lanes 1 - 4:
Western blot - Anti-Cyclin E1 antibody [EPR194] - BSA and Azide free (ab208695) at 1/1000 dilution

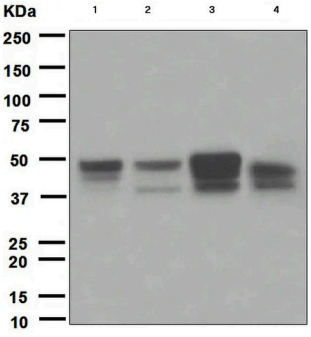
Lane 1:
Wild-type HAP1 whole cell lysate at 20 µg

Lane 2:
Cyclin E1 knockout HAP1 whole cell lysate at 20 µg

Lane 3:
HeLa whole cell lysate at 20 µg

Lane 4:
MCF7 whole cell lysate at 20 µg

Observed band size: 47 kDa



Western blot - Anti-Cyclin E1 antibody [EPR194] (ab133266)

All lanes:
Western blot - Anti-Cyclin E1 antibody [EPR194] (ab133266) at 1/1000 dilution

Lane 1:
HeLa cell lysate at 10 µg

Lane 2:
MCF7 cell lysate at 10 µg

Lane 3:
JAR cell lysate at 10 µg

Lane 4:
K562 cell lysate at 10 µg

Secondary

All lanes:
Goat anti-rabbit HRP conjugated antibody at 1/2000 dilution

Predicted band size: 47 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.