# abcam

## Product datasheet

# Anti-Cyclin El antibody [EPR194] ab133266





★★★★★ 3 Abreviews 17 References 8 Images

#### Overview

**Product name** Anti-Cyclin E1 antibody [EPR194]

**Description** Rabbit monoclonal [EPR194] to Cyclin E1

**Host species** Rabbit

**Tested applications** Suitable for: Flow Cyt (Intra), WB, ICC/IF

Unsuitable for: IHC-P

Species reactivity Reacts with: Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HeLa whole cell lysate (ab150035), MCF7 cell lysate, JAR cell lysate, K562 cell lysate and

Wild-type HAP1 whole cell lysate. ICC/IF: HeLa cells Flow Cyt (Intra): MCF7 cells

**General notes** 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.

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

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

#### **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 long

term. Avoid freeze / thaw cycle.

pH: 7.2 Storage buffer

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA

Purity Protein A purified

Clonality Monoclonal
Clone number EPR194
Isotype IgG

#### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab133266 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
Flow Cyt (Intra)		1/150.
WB	*****(1)	1/1000 - 1/10000. Detects a band of approximately 42-50 kDa (predicted molecular weight: 47 kDa).
ICC/IF	<b>★★★★</b> ★ ★ (2)	1/1000. For unpurified use at 1:500.

**Application notes** Is unsuitable for IHC-P.

# **Target**

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

**Tissue specificity** Highly expressed in testis and placenta. Low levels in bronchial epithelial cells.

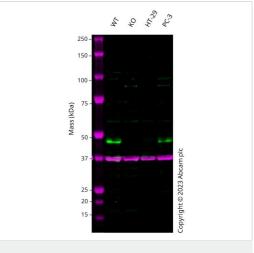
**Sequence similarities** Belongs to the cyclin family. Cyclin E subfamily.

Post-translational modifications

Phosphorylation of Thr-395 by GSK3 and of Ser-399 by CDK2 accelerates degradation via the ubiquitin proteasome pathway. Phosphorylated upon DNA damage, probably by ATM or ATR.

Cellular localization Nucleus.

# **Images**



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

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

Lane 1: Wild-type MCF7 cell lysate

Lane 2: CCNE1 knockout MCF7 cell lysate

Lane 3 : HT-29 cell lysate

Lane 4 : PC-3 cell lysate

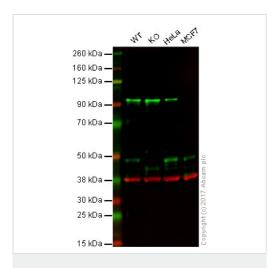
Lysates/proteins at 20 µg per lane.

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size:** 47 kDa **Observed band size:** 47 kDa

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.



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

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

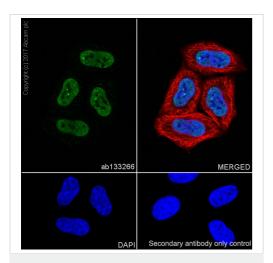
Lane 2: Cyclin E1 knockout HAP1 whole cell lysate (20  $\mu$ g)

Lane 3: HeLa whole cell lysate (20 µg)

Lane 4: MCF7 whole cell lysate (20 µg)

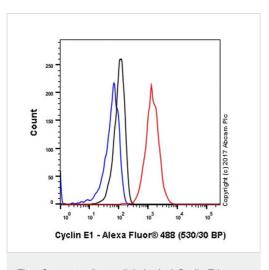
**Lanes 1 - 4:** Merged signal (red and green). Green - ab133266 observed at 47 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

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 <a href="mailto:ab9484">ab9484</a> (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 <a href="mailto:ab216773">ab216773</a> and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed <a href="mailto:ab216776">ab216776</a> secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

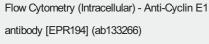


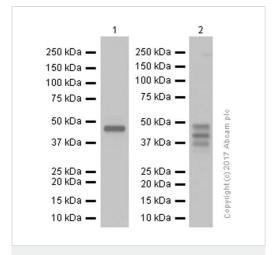
Immunocytochemistry/ Immunofluorescence - Anti-Cyclin E1 antibody [EPR194] (ab133266)

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 lgG(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.



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





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

**All lanes :** Anti-Cyclin E1 antibody [EPR194] (ab133266) at 1/10000 dilution (purified)

**Lane 1 :** HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

**Lane 2 :** JAR (Human placenta choriocarcinoma epithelial cell) whole cell lysates

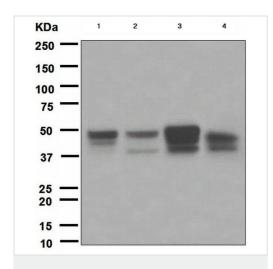
Lysates/proteins at 15 µg per lane.

# **Secondary**

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

Predicted band size: 47 kDa

Blocking and diluting buffer: 5% NFDM/TBST



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

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

Lane 1 : HeLa cell lysate
Lane 2 : MCF7 cell lysate
Lane 3 : JAR cell lysate

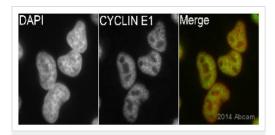
Lane 4: K562 cell lysate

Lysates/proteins at 10 µg per lane.

### **Secondary**

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

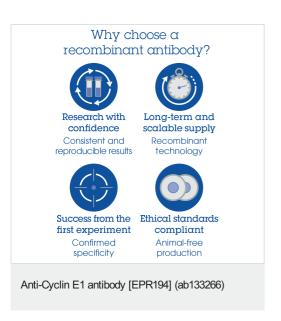
Predicted band size: 47 kDa



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

This image is courtesy of an Abreview submitted by Kirk McManus

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. <a href="mailto:ab150081">ab150081</a>, an Alexa Fluor<sup>®</sup> 488-conjugated goat anti-rabbit IgG polyclonal (1/200) was used as the secondary antibody. Counterstained with DAPI.



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