abcam

Product datasheet

Anti-Cyclin E2 antibody [EP454Y] ab40890

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Cyclin E2 antibody [EP454Y]
Description	Rabbit monoclonal [EP454Y] to Cyclin E2
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human Cyclin E2 aa 100-200. The exact sequence is proprietary.
Positive control	WB: Wild-type HAP1, MCF7, HeLa, and Jurkat (**ab7899**) whole cell lysates. ICC/IF: HeLa cells. IHC-P: Human breast carcinoma. Flow Cyt (intra): HeLa cells. IP: Jurkat whole cell lysate (**ab7899**).
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.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EP454Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab40890 in the following tested applications.

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

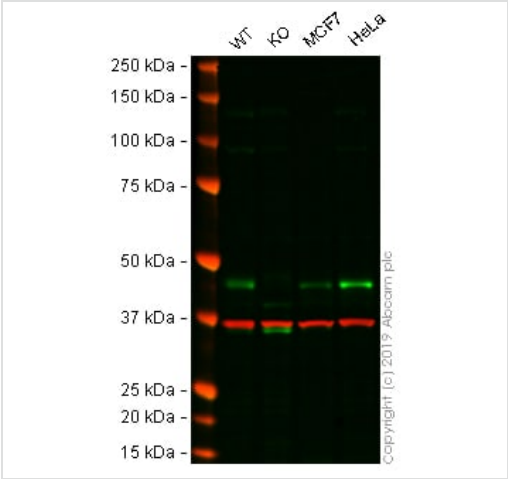
Application	Abreviews	Notes
Flow Cyt (Intra)		1/20.
WB		1/1000 - 1/10000. Detects a band of approximately 50 kDa (predicted molecular weight: 50 kDa).
IHC-P		1/100 - 1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
ICC/IF	★★★★★ **(1)**	1/50 - 1/250.
IP		1/20 - 1/50.

Target

Relevance The human Cyclin E2 gene encodes a 404 amino acid protein that is most closely related to Cyclin E. Cyclin E2 mRNA levels peaks at the G1 / S transition. Cyclin E2 associates with Cdk2 in a functional kinase complex that is inhibited by both p27 (Kip1) and p21 (Cip1). Cyclin E2 / Cdk2 phosphorylates histone H1 in vitro. G1 cyclin E controls the initiation of DNA synthesis by activating CDK2. Abnormally high levels of cyclin E expression have frequently been observed in human cancers. Unlike Cyclin E1, which is expressed in great majority of proliferating normal and neoplastically transformed cells, Cyclin E2 levels are low to undetectable in non transformed cells and increase significantly in neoplasm derived cells.

Cellular localization Nuclear

Images



Western blot - Anti-Cyclin E2 antibody [EP454Y] (ab40890)

All lanes : Anti-Cyclin E2 antibody [EP454Y] (ab40890) at 1/10000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : CCNE2 knockout HAP1 whole cell lysate
Lane 3 : MCF7 whole cell lysate
Lane 4 : HeLa whole cell lysate

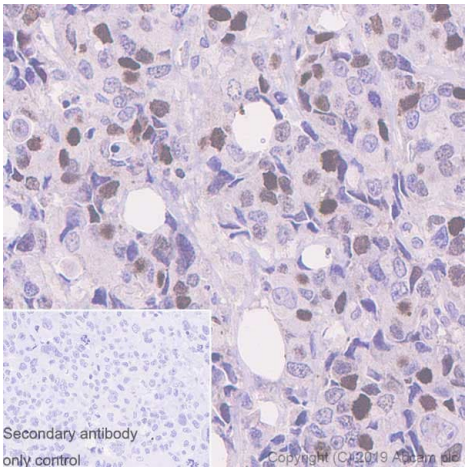
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 50 kDa
Observed band size: 45 kDa

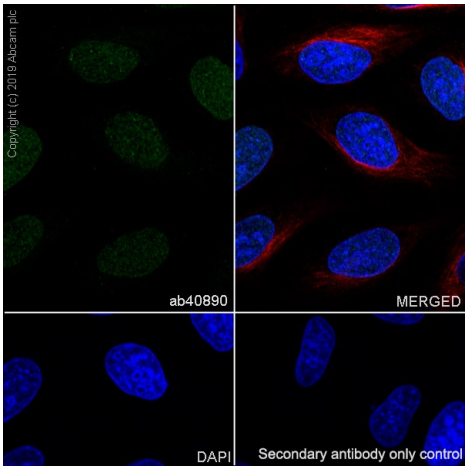
Lanes 1 - 4: Merged signal (red and green). Green - ab40890 observed at 45 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab40890 was shown to react with CCNE2 in HAP1 wild-type cells in Western blot. Loss of signal was observed when CCNE2 knockout sample was used. HAP1 wild-type and CCNE2 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% Milk in TBS-T (0.1% Tween®) before incubation with ab40890 and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 10000 dilution and a 1 in 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® 800CW) preabsorbed (**ab216772**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



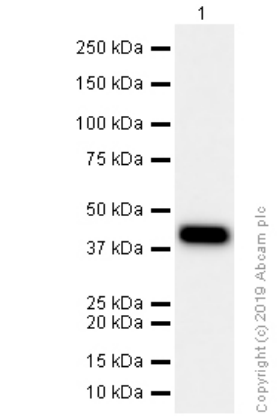
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin E2 antibody [EP454Y] (ab40890)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue sections labeling Cyclin E2 with purified ab40890 at 1/100 dilution (1.41 µg/ml). Heat mediated antigen retrieval was performed Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunocytochemistry/ Immunofluorescence - Anti-Cyclin E2 antibody [EP454Y] (ab40890)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Cyclin E2 with purified ab40890 at 1:50 dilution (2.8 µg/ml). Cells were fixed in 100% Methanol and permeabilized with None. 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 (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Western blot - Anti-Cyclin E2 antibody [EP454Y] (ab40890)

Anti-Cyclin E2 antibody [EP454Y] (ab40890) at 1/1000 dilution (Purified) + Jurkat (Human T cell leukemia T lymphocyte) whole cell lysates at 15 µg

Secondary

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

Predicted band size: 50 kDa

Observed band size: 45 kDa

Immunoprecipitation - Anti-Cyclin E2 antibody [EP454Y] (ab40890)

ab40890 (purified) at 1/20 dilution (1 µg) immunoprecipitating Cyclin E2 in Jurkat whole cell lysate.

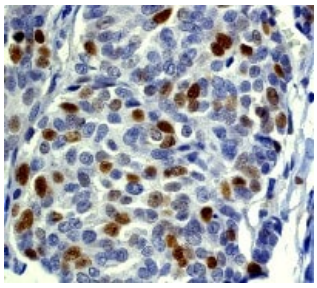
Lane 1 (input): Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate 10 µg

Lane 2 (+): ab40890 & Jurkat whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab40890 in Jurkat whole cell lysate

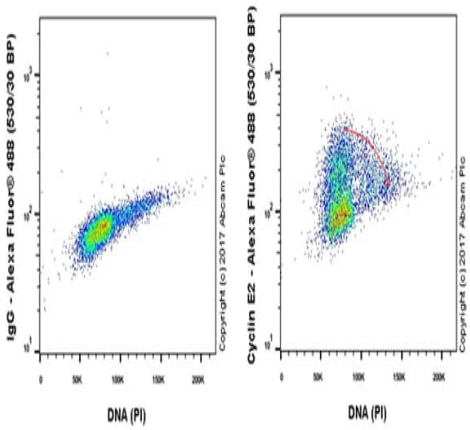
For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin E2 antibody [EP454Y] (ab40890)

ab40890 (unpurified) at 1/250 staining human breast carcinoma by immunohistochemistry, paraffin-embedded tissue.



Flow Cytometry (Intracellular) - Anti-Cyclin E2 antibody [EP454Y] (ab40890)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling Cyclin E2 (right) with purified ab40890 at a 1/20 dilution. Cells were fixed with 90% ethanol and permeabilized with 90% methanol. A goat anti-rabbit IgG (Alexa Fluorr® 488) (**ab150077**) was used as the secondary antibody at a 1/2000 dilution.

Left panel - Rabbit monoclonal IgG (**ab172730**).

Anti-Cyclin E2 antibody [EP454Y] (ab40890)

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