abcam

Product datasheet

Anti-Cyclin E1 antibody [EP435E] - BSA and Azide free ab208696

KO VALIDATED Recombinant RabMAb

★★★★★ 1 Abreviews 4 References 14 Images

Overview

Product name	Anti-Cyclin E1 antibody [EP435E] - BSA and Azide free
Description	Rabbit monoclonal [EP435E] to Cyclin E1 - BSA and Azide free
Host species	Rabbit
Specificity	This antibody recognises Cyclin E1. It is predicted to detect the splice isoform 2 based on sequence analysis.
Tested applications	**Suitable for:** Flow Cyt (Intra), IHC-P, WB, IP, ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1 and HeLa cell lysates, Human testis and placenta tissue lysates IP: HeLa cell lysate Flow Cyt (intra): HeLa and MCF7 Cells ICC/IF: HeLa cells IHC-P: Human placenta, Human colon carcinoma, wild type HAP-1.
General notes	ab208696 is the carrier-free version of **ab33911**. 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**.

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. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP435E
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab208696 in the following tested applications.

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

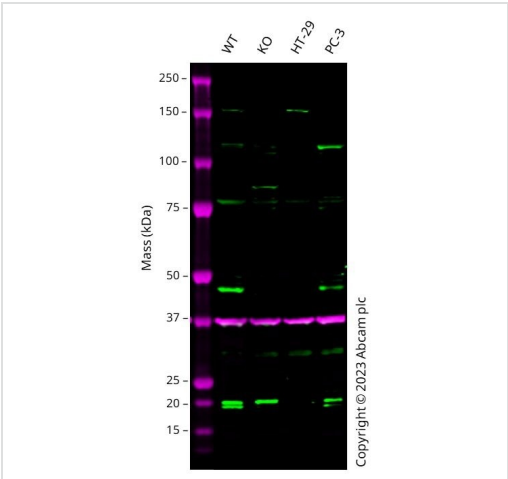
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 50 kDa (predicted molecular weight: 47 kDa).
IP		Use at an assay dependent concentration.
ICC/IF	★★★★★ (1)	Use at an assay dependent concentration.

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 [EP435E] - BSA and Azide free (ab208696)

All lanes : Anti-Cyclin E1 antibody [EP435E] (**ab33911**) 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.

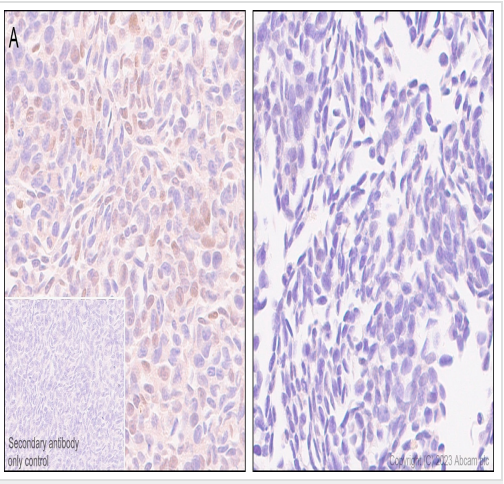
Developed using the ECL technique.

Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab33911**).
Western blot: Anti-CCNE1 antibody [EP435E] (**ab33911**) 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, **ab33911** 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.

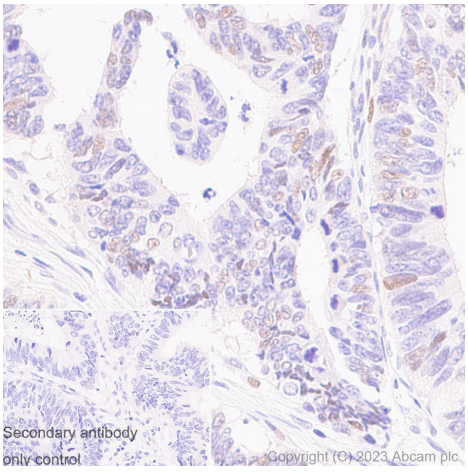


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin E1 antibody [EP435E] - BSA and Azide free (ab208696)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab33911**).

Immunohistochemical analysis of paraffin-embedded (A) Wild-type HAP1 (human chronic myelogenous leukemia near-haploid cell) cell pellets (B)CCNE1 KO HAP1 cell pellets tissue labeling Cyclin E1 with **ab33911** at 1/2000 (0.12 µg/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining on (A) Wild-type HAP1 cell pellets, no staining on (B) CCNE1 KO HAP1 cell pellets.The section was incubated with **ab33911** for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins

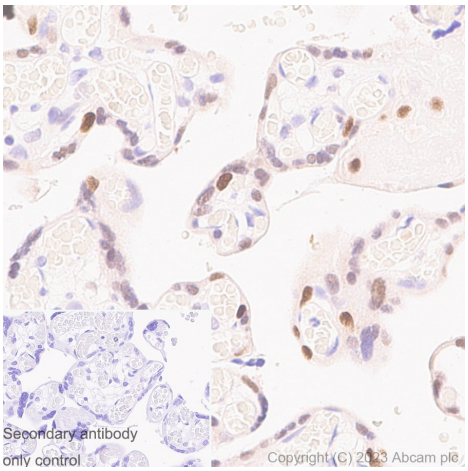


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin E1 antibody [EP435E] - BSA and Azide free (ab208696)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab33911**).

Immunohistochemical analysis of paraffin-embedded Human colon carcinoma tissue labeling Cyclin E1 with **ab33911** at 1/2000 (0.12 µg/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining on the human colon carcinoma. The section was incubated with **ab33911** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins

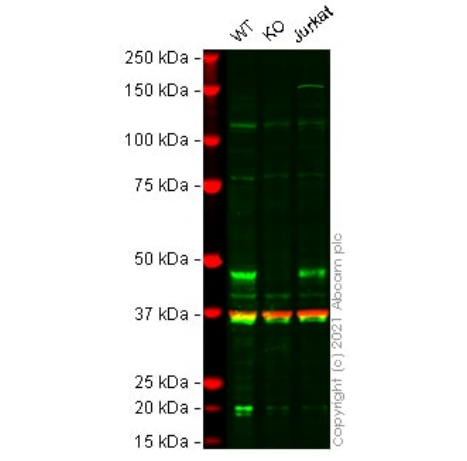


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin E1 antibody [EP435E] - BSA and Azide free (ab208696)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab33911**).

Immunohistochemical analysis of paraffin-embedded Human placenta tissue labeling Cyclin E1 with **ab33911** at 1/2000 (0.12 µg/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining on the human placenta. The section was incubated with **ab33911** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Western blot - Anti-Cyclin E1 antibody [EP435E] - BSA and Azide free (ab208696)

All lanes : Anti-Cyclin E1 antibody [EP435E] (**ab33911**) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : CCNE1 knockout HAP1 cell lysate
Lane 3 : Jurkat cell lysate

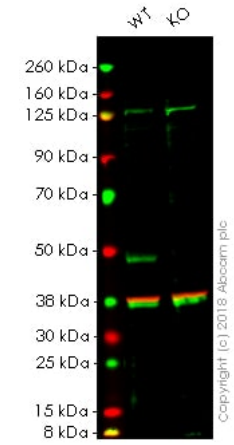
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

False colour image of Western blot: Anti-Cyclin E1 antibody [EP435E] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab33911** was

shown to bind specifically to Cyclin E1. A band was observed at 47 kDa in wild-type HAP1 cell lysates with no signal observed at this size in CCNE1 knockout cell line. To generate this image, wild-type and CCNE1 knockout HAP1 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 (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Anti-Cyclin E1 antibody [EP435E] - BSA and Azide free (ab208696)

All lanes : Anti-Cyclin E1 antibody [EP435E] (**ab33911**) at 1/1000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : CCNE1 (Cyclin E1) knockout HAP1 whole cell lysate

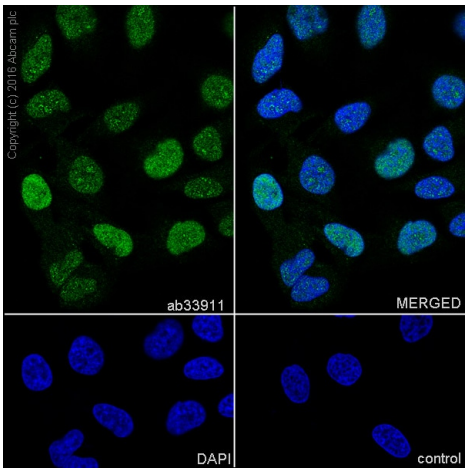
Lysates/proteins at 40 µg per lane.

Predicted band size: 47 kDa

Lanes 1 - 2: Merged signal (red and green). Green - **ab33911** observed at 47 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab33911 was shown to recognize CCNE1 in wild-type HAP1 cells as signal was lost at the expected MW in CCNE1 (Cyclin E1) knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and CCNE1 (Cyclin E1) knockout samples were subjected to SDS-PAGE. Ab33911 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab33911**) (unpurified).

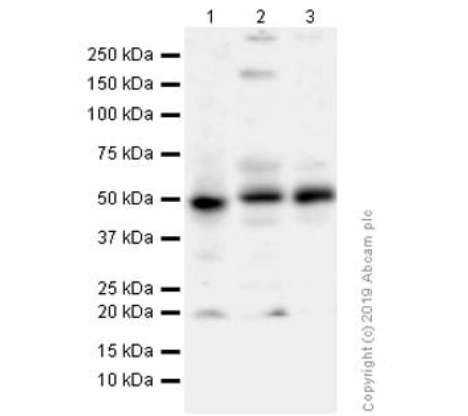


Immunocytochemistry/ Immunofluorescence - Anti-Cyclin E1 antibody [EP435E] - BSA and Azide free (ab208696)

Immunocytochemistry/Immunofluorescence analysis of HeLa (human cervix adenocarcinoma) cells labeling Cyclin E1 (green) with purified **ab33911** at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. Nuclei were counterstained with DAPI (blue).

Secondary Only Control: PBS was used instead of the primary antibody as the negative control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab33911**).



Western blot - Anti-Cyclin E1 antibody [EP435E] - BSA and Azide free (ab208696)

All lanes : Anti-Cyclin E1 antibody [EP435E] (**ab33911**) at 1/1000 dilution (Purified)

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : Human testis lysates
Lane 3 : Human placenta lysates

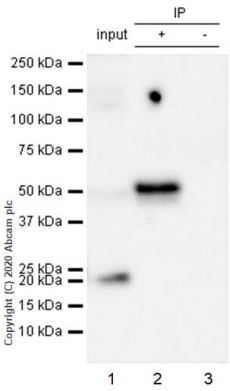
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Predicted band size: 47 kDa
Observed band size: 50 kDa

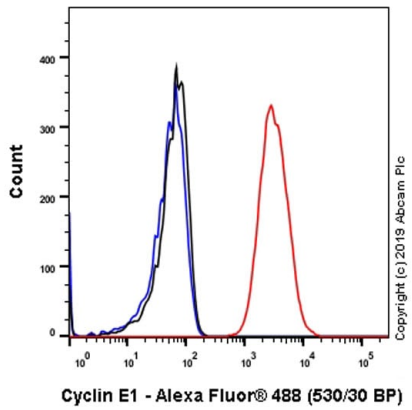
Cyclin E1 is highly expressed in testis and placenta which is described in PMID: 9840943.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab33911**).



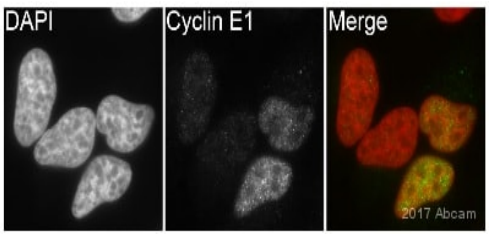
Immunoprecipitation - Anti-Cyclin E1 antibody [EP435E] - BSA and Azide free (ab208696)

Purified **ab33911** at 1/30 dilution (2ug) immunoprecipitating Cyclin E1 in HeLa whole cell lysate.
Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate (10µg)
Lane 2 (+): **ab33911** + HeLa whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab33911** in HeLa whole cell lysate.
VeriBlot for IP Detection Reagent (HRP) (**ab131366**) (1/1000) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 50 kDa
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab33911**).



Flow Cytometry (Intracellular) - Anti-Cyclin E1 antibody [EP435E] - BSA and Azide free (ab208696)

Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Cyclin E1 with Purified **ab33911** at 1/30 dilution (10 µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue). This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab33911**).

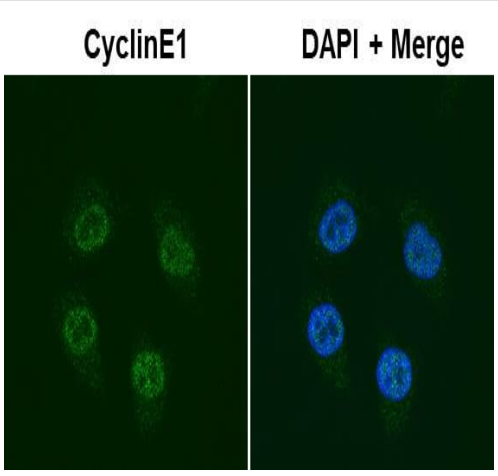


Immunocytochemistry/ Immunofluorescence - Anti-Cyclin E1 antibody [EP435E] - BSA and Azide free (ab208696)

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

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labeling Cyclin E1 with **ab33911** at 1/500 dilution. Cells were fixed in paraformaldehyde and permeabilized with 0.5% Triton X-100 in PBS. Staining with **ab33911** at 1/500 was carried out for 1 hour at 22°C in PBS buffer. **ab150081**, a Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed secondary antibody, was used at 1/200 dilution. DAPI was used to counterstain.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab33911**) (unpurified).

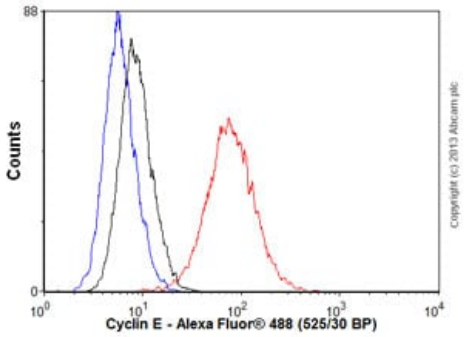


Immunocytochemistry/ Immunofluorescence - Anti-Cyclin E1 antibody [EP435E] - BSA and Azide free (ab208696)

This image is courtesy of an anonymous Abreview.

ab33911 staining Cyclin E1 in HeLa cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with 0.2% Triton X-100 and blocked with 2% BSA for 45 minutes at room temperature. Samples were incubated with primary antibody (1/300 in PBS + 2% BSA) for 14 hours at 4°C. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG polyclonal (1/500) was used as the secondary antibody.

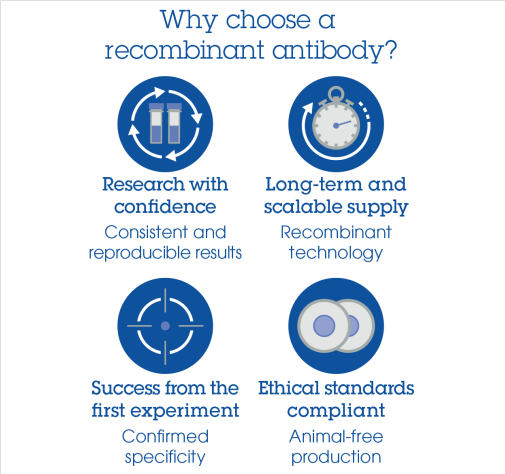
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab33911**) (unpurified).



Flow Cytometry (Intracellular) - Anti-Cyclin E1 antibody [EP435E] - BSA and Azide free (ab208696)

Overlay histogram showing MCF7 cells stained with **ab33911** (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (**ab33911**, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in MCF7 cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab33911**) (unpurified).



Anti-Cyclin E1 antibody [EP435E] - BSA and Azide free (ab208696)

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