abcam

Product datasheet

Anti-Cdk2 antibody [E304] ab32147

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Cdk2 antibody [E304]
Description	Rabbit monoclonal [E304] to Cdk2
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, IP, WB, IHC-P, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human Cdk2 aa 250 to the C-terminus (C terminal). The exact sequence is proprietary.
Epitope	The epitope is within the C-terminus of human Cdk2
Positive control	HeLa cells HeLa whole cell lysate (**ab150035**).
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**.

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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E304

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32147 in the following tested applications.

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

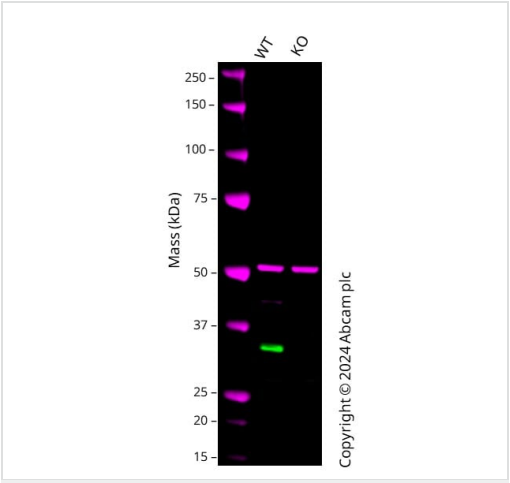
Application	Abreviews	Notes
ICC/IF	★★★★★ (3)	1/200. **For unpurified use at 1/100.**
IP		1/40.
WB	★★★★★ (5)	1/1000 - 1/10000. Detects a band of approximately 33 kDa (predicted molecular weight: 34 kDa).
IHC-P		1/50. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
Flow Cyt (Intra)		1/80. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target

Function Involved in the control of the cell cycle. Interacts with cyclins A, B1, B3, D, or E. Activity of CDK2 is maximal during S phase and G2.

Sequence similarities Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. CDC2/CDKX subfamily.
Contains 1 protein kinase domain.

Images



Western blot - Anti-Cdk2 antibody [E304] (ab32147)

All lanes : Anti-Cdk2 antibody [E304] (ab32147) at 1/2000 dilution

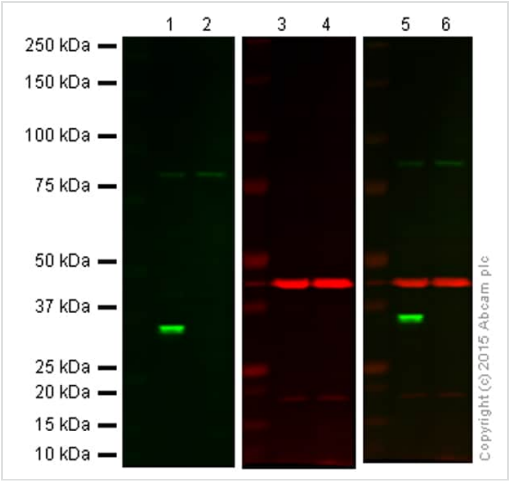
Lane 1 : Wild-type MCF7 cell lysate
Lane 2 : CDK2 knockout MCF7 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Predicted band size: 34 kDa
Observed band size: 34 kDa

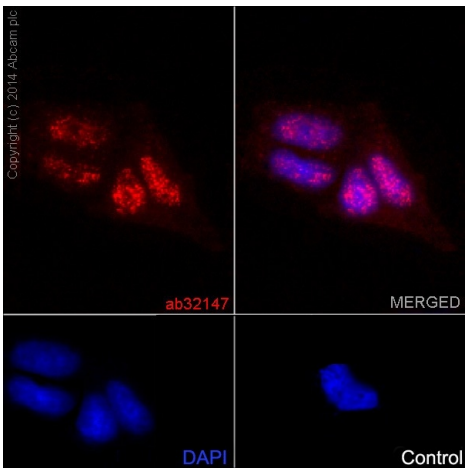
Western blot: Anti-CDK2 antibody [E304] (ab32147) staining at 1/2000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab32147 was shown to bind specifically to CDK2. A band was observed at 34 kDa in wild-type MCF7 cell lysates with no signal observed at this size in CDK2 knockout cell line **ab282628**. To generate this image, wild-type and CDK2 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-Cdk2 antibody [E304] (ab32147)

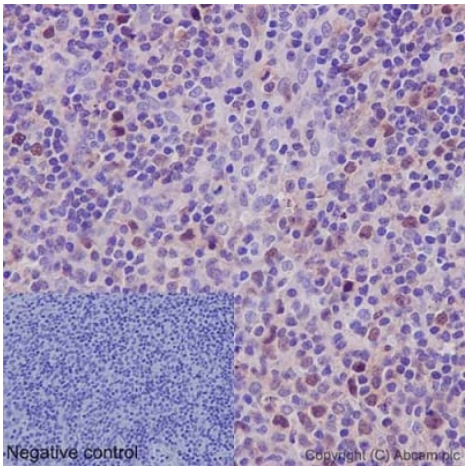
Lanes 1, 3 and 5: Wild-type HAP1 cell lysate (20 µg)
Lanes 2, 4 and 6: CDK2 knockout HAP1 cell lysate (20 µg)
Lanes 1 and 2: Green signal from target – ab32147 observed at 34 kDa
Lanes 3 and 4: Red signal from loading control – **ab8226** observed at 42 kDa
Lanes 5 and 6: Merged (red and green) signal

ab32147 was shown to specifically react with CDK2 when CDK2 knockout samples were used. Wild-type and CDK2 knockout samples were subjected to SDS-PAGE. ab32147 and **ab8226** (loading control to beta actin) were both diluted 1/1000 and incubated overnight at 4ºC. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging.



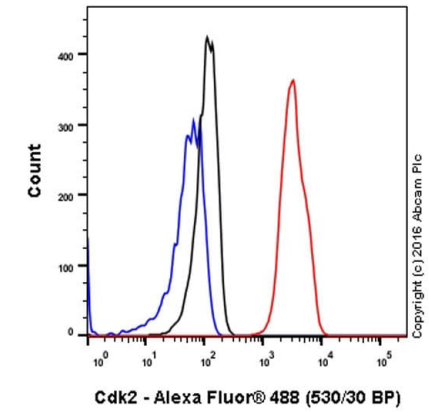
Immunocytochemistry/ Immunofluorescence - Anti-Cdk2 antibody [E304] (ab32147)

ab32147 staining Cdk2 in the HeLa cell line by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody (1/200). **ab150078** (1/500) an Alexa Fluor® 555-conjugated Goat anti-rabbit IgG was used as the secondary antibody. Nuclei were counterstained with DAPI.



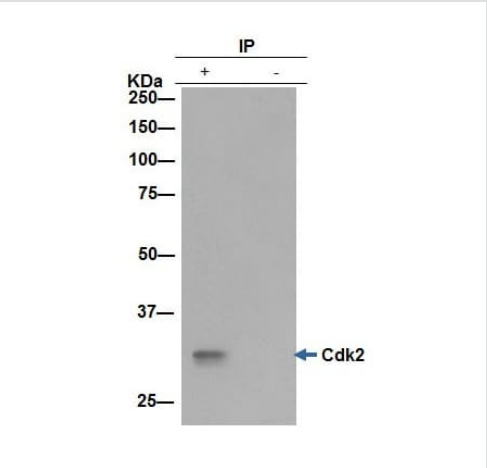
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cdk2 antibody [E304] (ab32147)

ab32147 staining Cdk2 in human tonsil tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed and paraffin-embedded, antigen retrieval was by heat mediation in Tris/EDTA buffer pH9. Samples were incubated with primary antibody (1/50). An undiluted HRP-conjugated mouse anti-rabbit IgG was used as the secondary antibody. Tissue counterstained with Hematoxylin. PBS was used in the negative control rather than the Primary antibody.



Flow Cytometry (Intracellular) - Anti-Cdk2 antibody [E304] (ab32147)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labelling Cdk2 with purified ab32147 at 1/80 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.

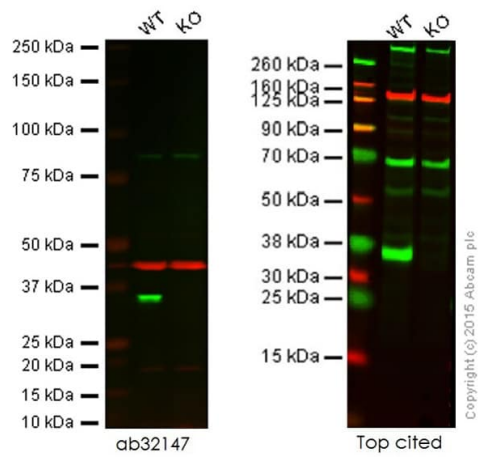


Immunoprecipitation - Anti-Cdk2 antibody [E304] (ab32147)

ab32147 (purified) at 1/40 immunoprecipitating Cdk2 from HeLa cells(Lane 1). Lane 2 - PBS. For western blotting, a HRP-conjugated anti-rabbit IgG, specific to the non-reduced form of IgG was used as the secondary antibody (1/1000).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

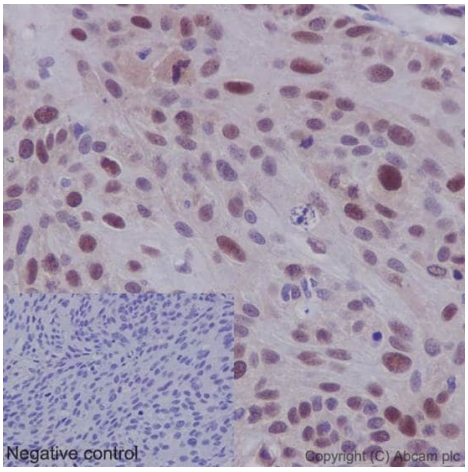


Western blot - Anti-Cdk2 antibody [E304] (ab32147)

Lanes 1: Wild-type HAP1 cell lysate (20 µg)
Lanes 2: CDK2 knockout HAP1 cell lysate (20 µg)

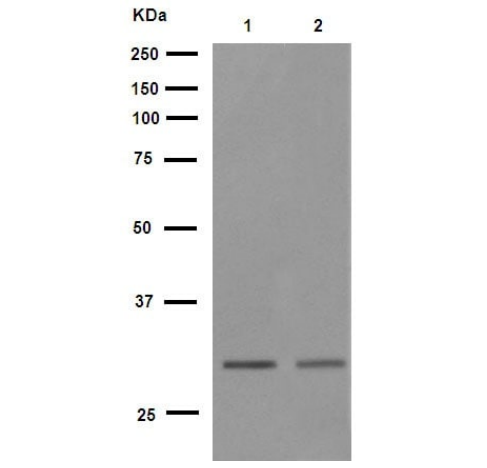
Lanes 1 - 2: Merged signal (red and green). Green - ab32147 observed at 34 kDa. Red - loading control, **ab8226**, observed at 42 kDa or **ab18058**, observed at 130 kDa.

This western blot image is a comparison between ab32147 and a competitor's top cited rabbit polyclonal antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cdk2 antibody [E304] (ab32147)

ab32147 staining Cdk2 in human squamous cell carcinoma of cervix tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed and paraffin-embedded, antigen retrieval was by heat mediation in Tris/EDTA buffer pH9. Samples were incubated with primary antibody (1/50). An undiluted HRP-conjugated mouse anti-rabbit IgG was used as the secondary antibody. Tissue counterstained with Hematoxylin. PBS was used in the negative control rather than the Primary antibody.



Western blot - Anti-Cdk2 antibody [E304] (ab32147)

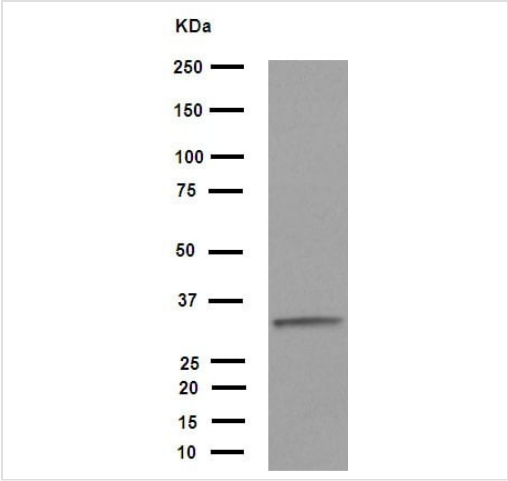
All lanes : Anti-Cdk2 antibody [E304] (ab32147) at 1/5000 dilution

Lane 1 : C6 cell lysate
Lane 2 : PC-12 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), HRP-conjugated at 1/1000 dilution

Predicted band size: 34 kDa



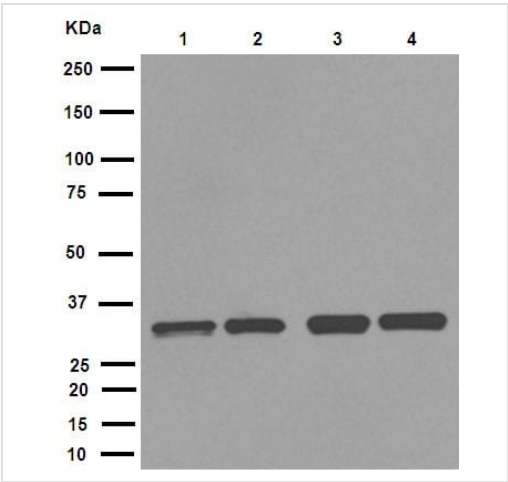
Western blot - Anti-Cdk2 antibody [E304] (ab32147)

Anti-Cdk2 antibody [E304] (ab32147) at 1/1000 dilution + NIH/3T3 cell lysate at 20 µg

Secondary

Goat Anti-Rabbit IgG, (H+L), HRP-conjugated at 1/1000 dilution

Predicted band size: 34 kDa



Western blot - Anti-Cdk2 antibody [E304] (ab32147)

All lanes : Anti-Cdk2 antibody [E304] (ab32147) at 1/1000 dilution

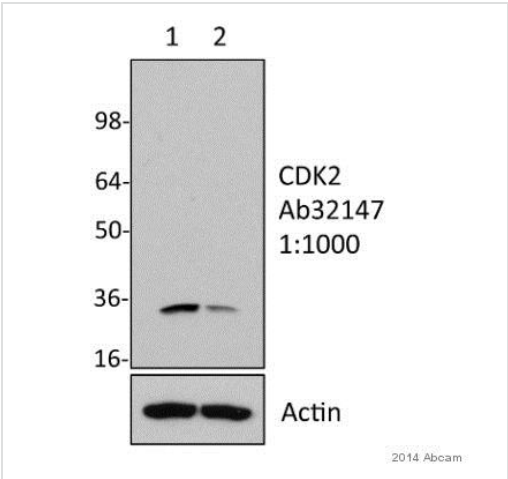
Lane 1 : Jurkat cell lysate
Lane 2 : Hela cell lysate
Lane 3 : K562 cell lysate
Lane 4 : 293 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG, (H+L), HRP-conjugated at 1/1000 dilution

Predicted band size: 34 kDa



Western blot - Anti-Cdk2 antibody [E304] (ab32147)

This image is courtesy of an Abreview submitted by Sonia Rocha

All lanes : Anti-Cdk2 antibody [E304] (ab32147) at 1/1000 dilution (unpurified)

Lane 1 : Human osteosarcoma whole cell lysate - control, non-targeting siRNA
Lane 2 : Human osteosarcoma whole cell lysate - siRNA for CDK2

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : HRP-conjugated goat anti-rabbit IgG polyclonal at 1/2000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 34 kDa
Observed band size: 34 kDa

Exposure time: 2 seconds

Western blot - Anti-Cdk2 antibody [E304] (ab32147)

All lanes : Anti-Cdk2 antibody [E304] (ab32147) at 1/1000 dilution (unpurified)

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate (**ab27252**) at 10 µg
Lane 2 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate (**ab27252**) at 20 µg

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution

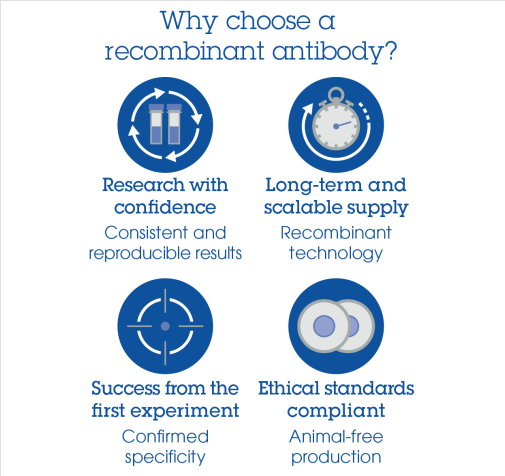
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 34 kDa
Observed band size: 34 kDa

Exposure time: 4 minutes

This blot was produced using a 10% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 5% Bovine Serum Albumin before being incubated with ab32147 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution.



Anti-Cdk2 antibody [E304] (ab32147)

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