abcam

Product datasheet

Anti-CDK1 + Cdk2 + Cdk3 (phospho T14) antibody [E161] ab32384

Recombinant RabMAb

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Overview

Product name	Anti-CDK1 + Cdk2 + Cdk3 (phospho T14) antibody [E161]
Description	Rabbit monoclonal [E161] to CDK1 + Cdk2 + Cdk3 (phospho T14)
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, IP, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Cow
Immunogen	Synthetic peptide within Human CDK1 aa 1-100. The exact sequence is proprietary. Database link: **P06493**
Positive control	WB: HeLa cell lysate. IHC-P: Human lymphoma.
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 (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	E161
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32384 in the following tested applications.

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

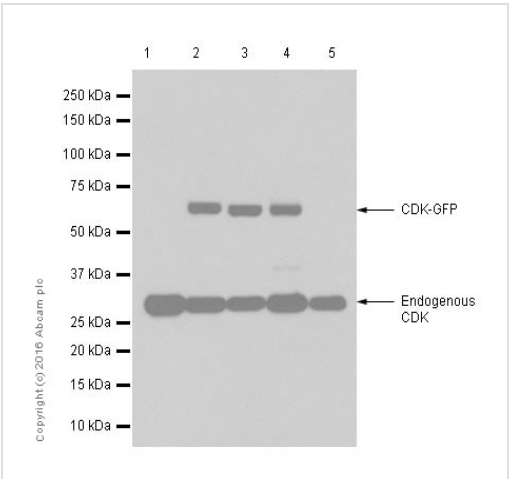
Application	Abreviews	Notes
WB	★★★★★ **(1)**	1/1000 - 1/10000. Detects a band of approximately 34 kDa (predicted molecular weight: 34 kDa).
IHC-P		1/50 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
IP		1/50.
ICC/IF		1/100 - 1/500.

Target

Cellular localization CDK1: Nucleus.

Form CDK1: CDK1 can be located to the Nucleus, cytoplasm and Mithocondria. It's cytoplasmic during interphase and reversibly translocated from cytoplasm to the nucleus when phosphorilated before G2-M transition when associated with cyclin-B1. Accumulates in mitochondria in G2-arrested cells upon DNA-damage.

Images



Western blot - Anti-CDK1 + Cdk2 + Cdk3 (phospho T14) antibody [E161] (ab32384)

All lanes : Anti-CDK1 + Cdk2 + Cdk3 (phospho T14) antibody [E161] (ab32384) at 1/2000 dilution (purified)

Lane 1 : Empty vector transfected 293T (human embryonic kidney) whole cell lysates

Lane 2 : CDK1-GFP transfected 293T (human embryonic kidney) whole cell lysates

Lane 3 : CDK2-GFP transfected 293T (human embryonic kidney) whole cell lysates

Lane 4 : CDK3-GFP transfected 293T (human embryonic kidney) whole cell lysates

Lane 5 : CDK5-GFP transfected 293T (human embryonic kidney) whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary

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

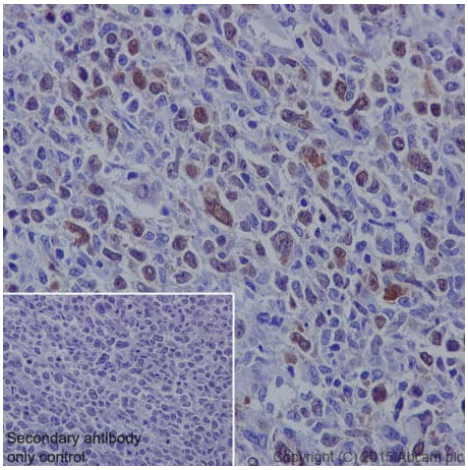
Predicted band size: 34 kDa

Observed band size: 62, 34 kDa

Exposure time: 15 seconds

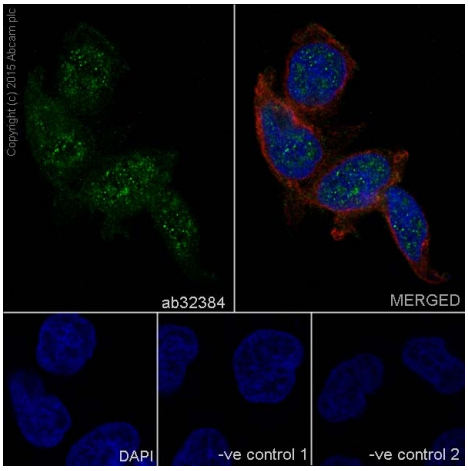
Blocking/Diluting buffer 5% NFDM/TBST

34kDa band represents endogenous CDK, and 62kDa represents CDK-GFP.



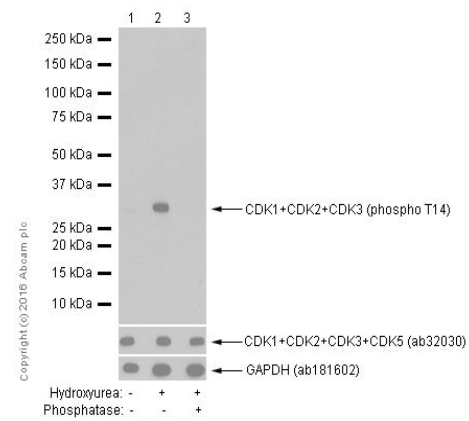
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CDK1 + Cdk2 + Cdk3 (phospho T14) antibody [E161] (ab32384)

Immunohistochemical staining of paraffin embedded human B cell lymphoma with purified ab32384 at a working dilution of 1/500. The secondary antibody used is HRP goat anti-rabbit IgG H&L (**ab97051**) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Immunocytochemistry/ Immunofluorescence - Anti-CDK1 + Cdk2 + Cdk3 (phospho T14) antibody [E161] (ab32384)

Immunofluorescence staining of HeLa cells with purified ab32384 at a working dilution of 1/200, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (**ab150077**), used at a dilution of 1/1000. **ab7291**, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with **ab150120** (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab32384 was used at a dilution of 1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody (**ab150120**) at a dilution of 1/500. For negative control 2, **ab7291** (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody (**ab150077**) at a dilution of 1/400.



Western blot - Anti-CDK1 + Cdk2 + Cdk3 (phospho T14) antibody [E161] (ab32384)

All lanes : Anti-CDK1 + Cdk2 + Cdk3 (phospho T14) antibody [E161] (ab32384) at 1/2000 dilution (purified)

Lane 1 : Untreated HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysates
Lane 2 : HeLa (Human epithelial cell line from cervix adenocarcinoma) treated with 3mM hydroxyurea for 20 hours whole cell lysates
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) treated with 3mM hydroxyurea for 20 hours whole cell lysates. Then the membrane was incubated with phosphatase.

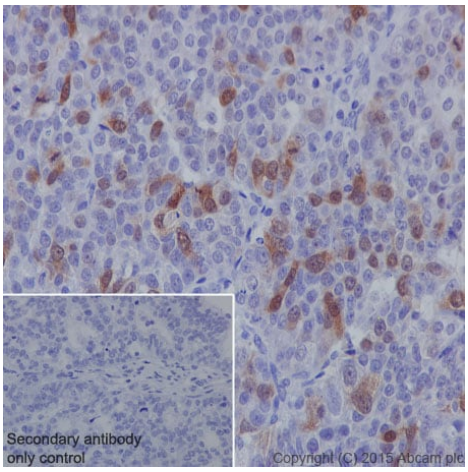
Lysates/proteins at 15 µg per lane.

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

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

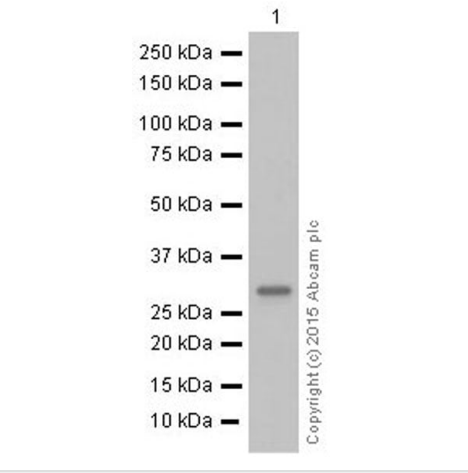
Exposure time: 30 seconds

Blocking/Diluting buffer



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CDK1 + Cdk2 + Cdk3 (phospho T14) antibody [E161] (ab32384)

Immunohistochemical staining of paraffin embedded human ovarian carcinoma with purified ab32384 at a working dilution of 1/500. The secondary antibody used is HRP goat anti-rabbit IgG H&L (**ab97051**) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



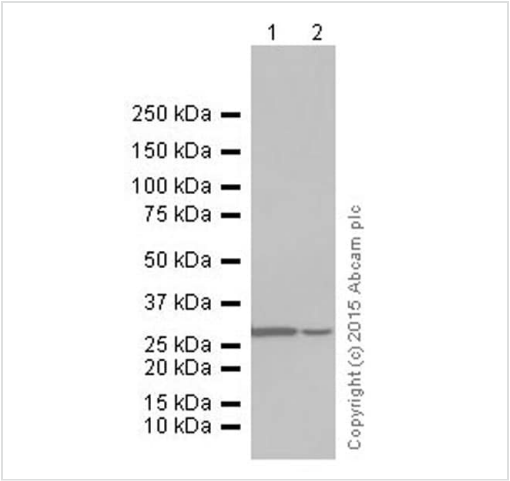
Western blot - Anti-CDK1 + Cdk2 + Cdk3 (phospho T14) antibody [E161] (ab32384)

Anti-CDK1 + Cdk2 + Cdk3 (phospho T14) antibody [E161] (ab32384) at 1/5000 dilution (purified) + Raw264.7 cell lysate at 10 µg

Secondary
HRP goat anti-rabbit IgG (H+L) at 1/20000 dilution

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

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST



Western blot - Anti-CDK1 + Cdk2 + Cdk3 (phospho T14) antibody [E161] (ab32384)

All lanes : Anti-CDK1 + Cdk2 + Cdk3 (phospho T14) antibody [E161] (ab32384) at 1/10000 dilution (purified)

Lane 1 : C6 cell lysate

Lane 2 : PC-12 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

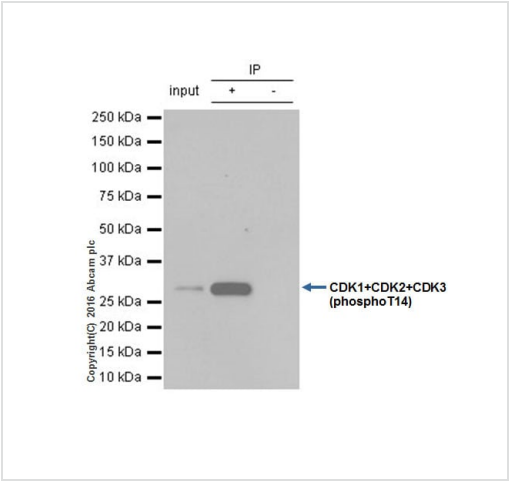
All lanes : HRP goat anti-rabbit IgG (H+L) at 1/20000 dilution

Predicted band size: 34 kDa

Observed band size: 34 kDa

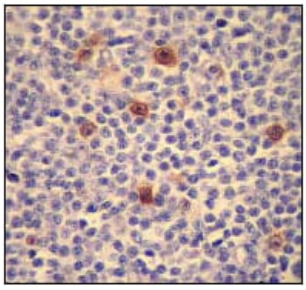
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



Immunoprecipitation - Anti-CDK1 + Cdk2 + Cdk3 (phospho T14) antibody [E161] (ab32384)

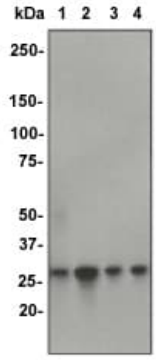
ab32384 (purified) at 1/50 immunoprecipitating CDK1 in 10 µg HEK293 (Lanes 1 and 2, observed at 34 kDa). Lane 3 - PBS. For western blotting, HRP Veriblot for IP Detection Reagent (**ab131366**) was used for detection (1/10 000). Blocking buffer and concentration: 5% NFDM/TBST Dilution buffer and concentration: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CDK1 + Cdk2 + Cdk3 (phospho T14) antibody [E161] (ab32384)

Unpurified ab32384, at a 1/50 dilution, staining Cdc2 in paraffin embedded human lymphoma tissue sections by Immunohistochemistry.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Western blot - Anti-CDK1 + Cdk2 + Cdk3 (phospho T14) antibody [E161] (ab32384)

All lanes : Anti-CDK1 + Cdk2 + Cdk3 (phospho T14) antibody [E161] (ab32384) at 1/1000 dilution (unpurified)

Lane 1 : A431 cell lysate
Lane 2 : HepG2 cell lysate
Lane 3 : SKBR3 cell lysate
Lane 4 : HeLa cell lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 34 kDa

Anti-CDK1 + Cdk2 + Cdk3 (phospho T14) antibody [E161] (ab32384)

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