abcam

Product datasheet

Anti-Cdc25C antibody [E302] ab32444

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Cdc25C antibody [E302]
Description	Rabbit monoclonal [E302] to Cdc25C
Host species	Rabbit
Specificity	The antibody can also detect splice isoform 2, 4 and 5 of human Cdc25C, based on sequence homology.
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, Hap1, K562 and HEK293 cell lysates. IHC-P: Human pancreas and urinary bladder carcinoma tissue. ICC/IF: HeLa cells. IP: HeLa cells. Flow Cyt (intra): HeLa 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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	E302
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32444 in the following tested applications.

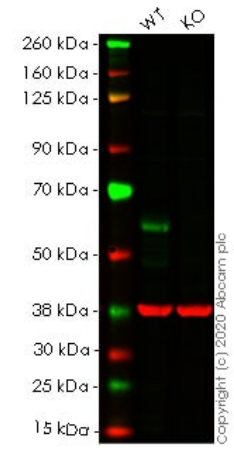
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB	★★★★☆ **(2)**	1/1000 - 1/5000. Detects a band of approximately 60 kDa (predicted molecular weight: 53 kDa).
IHC-P		1/2500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. **For unpurified, use 1/250 - 1/500.**
ICC/IF		1/250 - 1/500.
IP		1/30 - 1/80.

Target

Function	Functions as a dosage-dependent inducer in mitotic control. It is a tyrosine protein phosphatase required for progression of the cell cycle. It directly dephosphorylates CDK1 and activate its kinase activity.
Sequence similarities	Belongs to the MPI phosphatase family. Contains 1 rhodanese domain.
Developmental stage	Expressed predominantly in G2 phase.
Post-translational modifications	Phosphorylated by CHK1 on Ser-216. This phosphorylation creates a binding site for 14-3-3 protein and inhibits the phosphatase. Phosphorylated by PLK4.
Cellular localization	Nucleus.

Images



Western blot - Anti-Cdc25C antibody [E302] (ab32444)

All lanes : Anti-Cdc25C antibody [E302] (ab32444) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : CDC25C knockout HeLa cell lysate

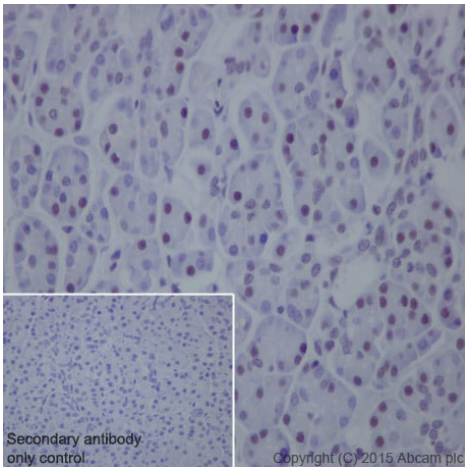
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 53 kDa
Observed band size: 58 kDa

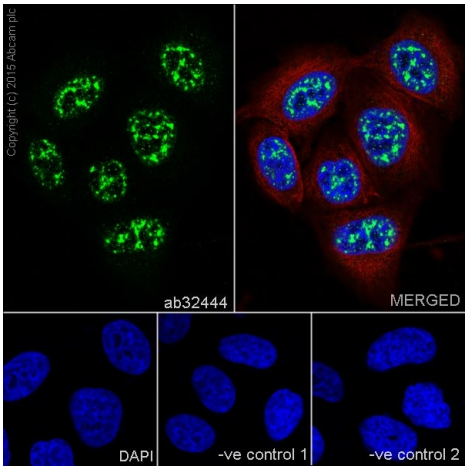
Lanes 1- 2: Merged signal (red and green). Green - ab32444 observed at 58 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab32444 was shown to react with Cdc25C in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab265189** (knockout cell lysate **ab257387**) was used. Wild-type HeLa and CDC25C knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab32444 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. 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 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cdc25C antibody [E302] (ab32444)

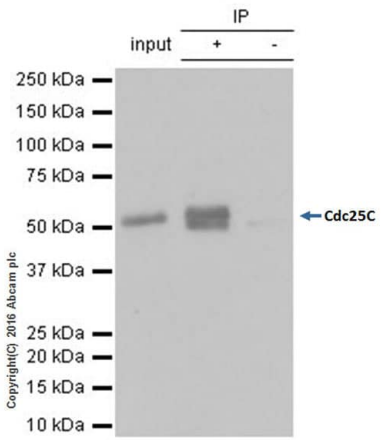
Immunohistochemical analysis of paraffin embedded human pancreas tissue section labelling Cdc25C with purified ab32444 at dilution of 1/2500. The secondary antibody used was Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**), at dilution of 1/500. The sample was counter-stained with hematoxylin. Antigen retrieval 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-Cdc25C antibody [E302] (ab32444)

Immunocytochemistry/Immunofluorescence analysis of HeLa (human cervix adenocarcinoma) cells labelling Cdc25C with purified ab32444 at 1/400. 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. The cells were co-stained with **ab7291**, a mouse anti-tubulin antibody (1/1000) using **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) as the secondary. Nuclei couterstained with DAPI (blue).

For negative control 1, rabbit primary antibody was used, followed by anti-mouse secondary antibody (**ab150120**). For negative control 2, mouse primary antibody (**ab7291**) was used followed by anti-rabbit secondary antibody (**ab150077**).



Immunoprecipitation - Anti-Cdc25C antibody [E302] (ab32444)

Ab32444 (purified) at 1/30 immunoprecipitating Cdc25C in HeLa (human cervix adenocarcinoma) whole cell lysate.

Lane 1 (input): HeLa (human cervix adenocarcinoma) whole cell lysate

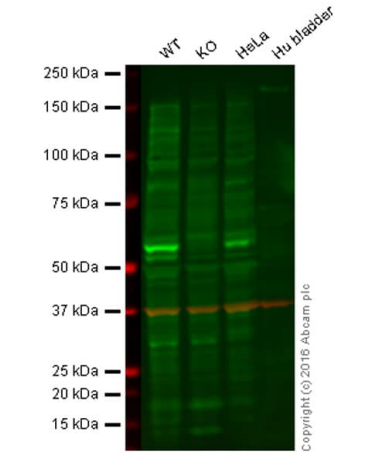
Lane 2 (+): ab32444 + HeLa (human cervix adenocarcinoma) whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32444 in HeLa (human cervix adenocarcinoma) whole cell lysate

For western blotting, **ab131366** VeriBlot for IP Detection Reagent (HRP) was used for detection (1/10000).

Blocking buffer and concentration: 5% NFDM/TBST.

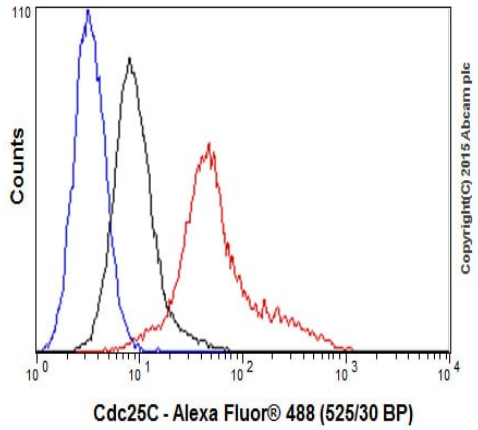
Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-Cdc25C antibody [E302] (ab32444)

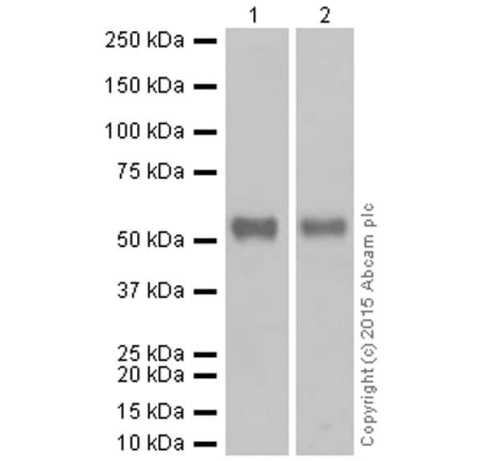
Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: Cdc25C knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: Hu bladder cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab32444 observed at 55 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab32444 was shown to recognize Cdc25C when Cdc25C knockout samples were used, along with additional cross-reactive bands. Wild-type and Cdc25C knockout samples were subjected to SDS-PAGE. ab32444 and **ab8245** (loading control to GAPDH) were diluted at 1/2500 and 1/10 000 respectively and incubated overnight at 4°C. 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 1/10000 dilution for 1 h at room temperature before imaging.



Flow Cytometry (Intracellular) - Anti-Cdc25C antibody [E302] (ab32444)

Intracellular Flow Cytometry analysis of K562 (human chronic myelogenous leukemia) cells labelling Cdc25C with purified ab32444 at 1/180 (red). Cells were fixed with 4% paraformaldehyde. Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Western blot - Anti-Cdc25C antibody [E302] (ab32444)

All lanes : Anti-Cdc25C antibody [E302] (ab32444) at 1/5000 dilution (purified)

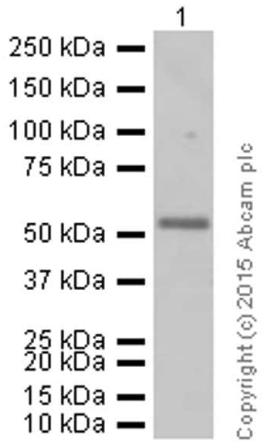
Lane 1 : K562 (human chronic myelogenous leukemia) whole cell lysate
Lane 2 : HEK293 (human embryonic kidney) whole cell lysates

Lysates/proteins at 20 µg per lane.

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

Predicted band size: 53 kDa
Observed band size: 60 kDa

Blocking and diluting buffer 5% NFDM/TBST



Western blot - Anti-Cdc25C antibody [E302] (ab32444)

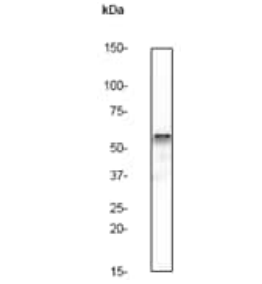
Anti-Cdc25C antibody [E302] (ab32444) at 1/1000 dilution (purified) + HeLa (human cervix adenocarcinoma) whole cell lysate at 20 µg

Secondary

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

Predicted band size: 53 kDa
Observed band size: 60 kDa

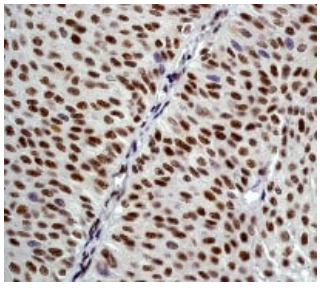
Blocking and diluting buffer 5% NFDM/TBST



Western blot - Anti-Cdc25C antibody [E302] (ab32444)

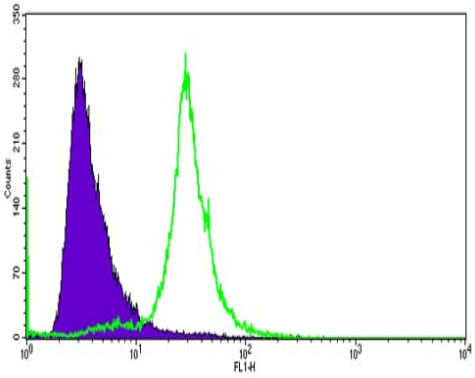
Anti-Cdc25C antibody [E302] (ab32444) at 1/5000 dilution (unpurified) + HeLa cell lysate

Predicted band size: 53 kDa
Observed band size: 60 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cdc25C antibody [E302] (ab32444)

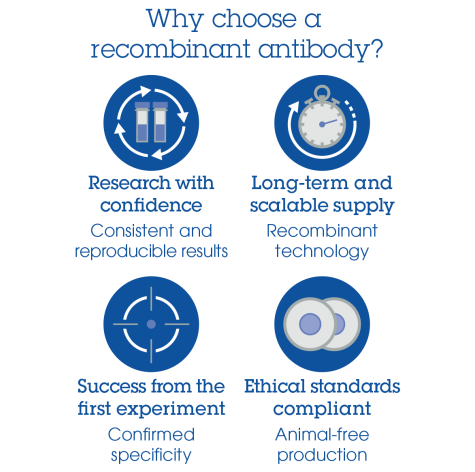
Immunohistochemical analysis of paraffin-embedded human urinary bladder carcinoma unpurified ab32444 at 1/250 dilution.



Flow Cytometry (Intracellular) - Anti-Cdc25C antibody [E302] (ab32444)

This image is courtesy of an Abreview submitted by Dr Brandon White

Intracellular Flow Cytometry analysis of HeLa cells, staining Cdc25C with unpurified ab32444. Cells were fixed with formaldehyde and permeabilized with 90% methanol. Samples were incubated with primary antibody (1/20 in PBS + 10% goat serum) for 1 hour at 23°C. A FITC-conjugated goat anti-rabbit polyclonal IgG (1/1000) was used as the secondary antibody.



Anti-Cdc25C antibody [E302] (ab32444)

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