abcam

Product datasheet

Anti-Cdc25C antibody [E302] - BSA and Azide free ab232553

KO 評価済 リコンビナント RabMAb

画像数 9

製品の概要

製品名	Anti-Cdc25C antibody [E302] - BSA and Azide free
製品の詳細	Rabbit monoclonal [E302] to Cdc25C - BSA and Azide free
由来種	Rabbit
特異性	The antibody can also detect splice isoform 2, 4 and 5 of human Cdc25C, based on sequence homology.
アプリケーション	**適用あり:** WB, IP, IHC-P, ICC/IF, Flow Cyt (Intra)
種交差性	**交差種:** Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: HAP1 and HeLa cell lysate. IHC-P: Human pancreas and urinary bladder tissues. IP: HeLa cell lysate. Flow Cyt (intra): K562 and HeLa cells. IHC-P: HeLa cells.
特記事項	ab232553 is the carrier-free version of **ab32444**. 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.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C. Do Not Freeze.
バッファー	pH: 7.2 Constituent: PBS
キャリア・フリー	はい
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	E302
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab232553の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

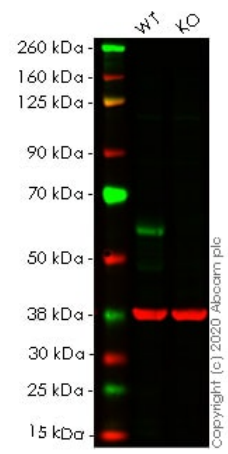
アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Predicted molecular weight: 53 kDa.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.

ターゲット情報

機能	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.
配列類似性	Belongs to the MPI phosphatase family. Contains 1 rhodanese domain.
発生段階	Expressed predominantly in G2 phase.
翻訳後修飾	Phosphorylated by CHK1 on Ser-216. This phosphorylation creates a binding site for 14-3-3 protein and inhibits the phosphatase. Phosphorylated by PLK4.

細胞内局在	Nucleus.

画像



Western blot - Anti-Cdc25C antibody [E302] - BSA and Azide free (ab232553)

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.

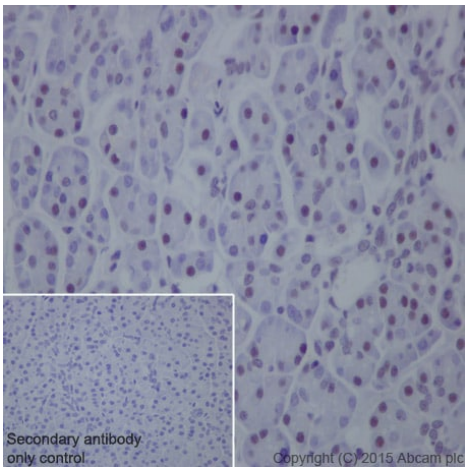
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (**ab32444**).

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.

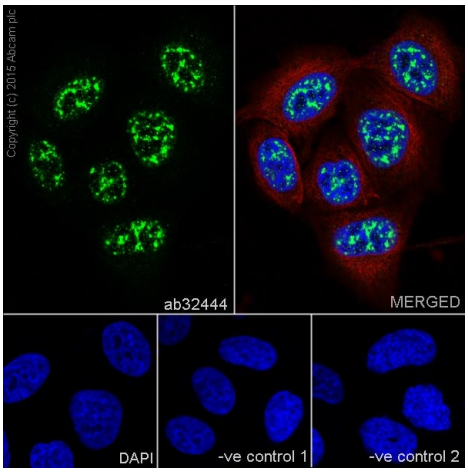
ab32444 was shown to react with Cdc25C in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab265189** (knockout cell lysate **ab257387**) was used. Wild-type HeLa and CDC25C knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour 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] - BSA and Azide free (ab232553)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32444**).

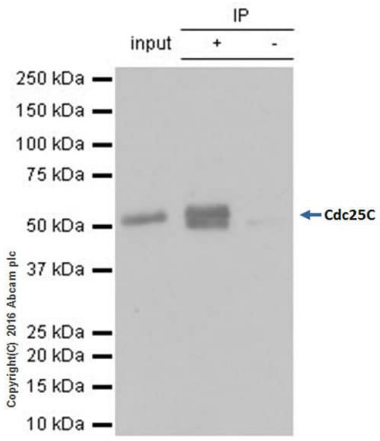


Immunocytochemistry/ Immunofluorescence - Anti-Cdc25C antibody [E302] - BSA and Azide free (ab232553)

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**).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32444**).



Immunoprecipitation - Anti-Cdc25C antibody [E302] - BSA and Azide free (ab232553)

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

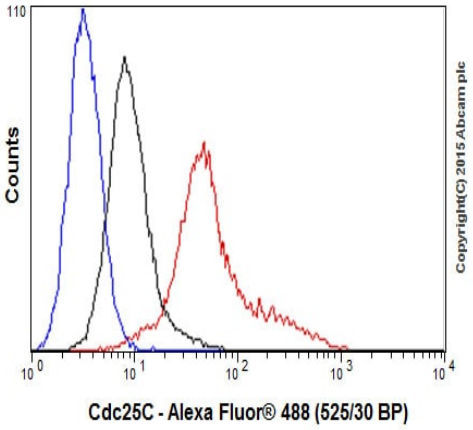
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab32444** in HeLa (human cervix adenocarcinoma) whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1000 dilution.

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

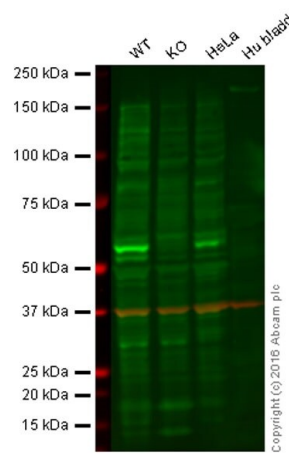
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32444**).



Flow Cytometry (Intracellular) - Anti-Cdc25C antibody [E302] - BSA and Azide free (ab232553)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32444**).

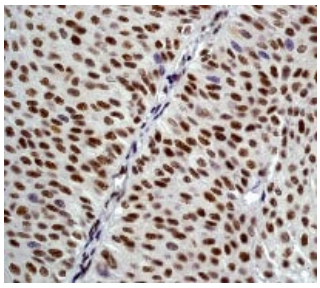


Western blot - Anti-Cdc25C antibody [E302] - BSA and Azide free (ab232553)

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: Human 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 1/2,500 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 1/10,000 dilution for 1 hour at room temperature before imaging.

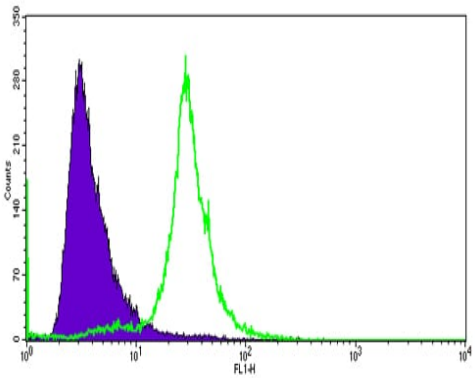
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32444**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cdc25C antibody [E302] - BSA and Azide free (ab232553)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32444**).

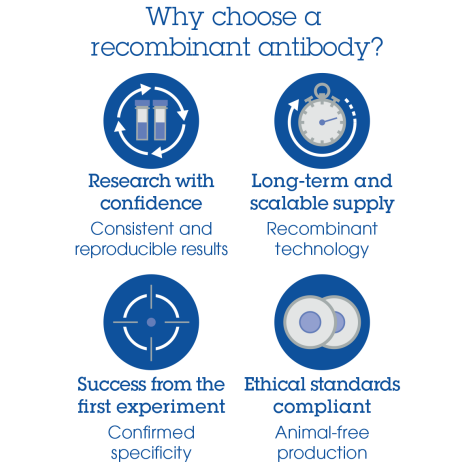


Flow Cytometry (Intracellular) - Anti-Cdc25C antibody [E302] - BSA and Azide free (ab232553)

This image is courtesy of an Abreview submitted by 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32444**).



Anti-Cdc25C antibody [E302] - BSA and Azide free (ab232553)

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