abcam

Product datasheet

Anti-CDC42 antibody ab64533



★★★★★★ 8 Abreviews 40 References 5 Images

Overview

Product name Anti-CDC42 antibody

Description Rabbit polyclonal to CDC42

Host species Rabbit

Specificity Replenishment batches of our polyclonal antibody, ab64533 are tested in WB. Previous batches

> were additionally validated in ICC/IF. This application is still expected to work and is covered by our Abpromise guarantee. You may also be interested in our alternative recombinant antibody,

ab187643.

Tested applications Suitable for: ICC/IF, WB

Species reactivity Reacts with: Mouse, Human

Predicted to work with: Rat, Pig, Saccharomyces cerevisiae, Chimpanzee, Zebrafish

Immunogen Synthetic peptide conjugated to KLH derived from within residues 100 to the C-terminus of

Human CDC42.Read Abcam's proprietary immunogen policy(Peptide available as ab66571.)

General notes The Life Science industry has been in the grips of a reproducibility crisis for a number of years.

> Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

Properties

Form

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -

80°C. Avoid freeze / thaw cycle.

Storage buffer pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising

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agent. If you would like information about the formulation of a specific lot, please contact our

scientific support team who will be happy to help.

Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab64533 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	★★★ ☆☆ <u>(1)</u>	Use a concentration of 5 µg/ml.
WB	★★★★☆ (4)	Use a concentration of 1 µg/ml. Detects a band of approximately 21 kDa (predicted molecular weight: 21 kDa).

Target	
Function	Plasma membrane-associated small GTPase which cycl

Plasma membrane-associated small GTPase which cycles between an active GTP-bound and an
inactive GDP-bound state. In active state binds to a variety of effector proteins to regulate cellular
responses. Involved in epithelial cell polarization processes. Causes the formation of thin, actin-

rich surface projections called filopodia.

Sequence similaritiesBelongs to the small GTPase superfamily. Rho family. CDC42 subfamily.

Post-translationalAMPylation at Tyr-32 and Thr-35 are mediated by bacterial enzymes in case of infection bymodificationsH.somnus and V.parahaemolyticus, respectively. AMPylation occurs in the effector region and

leads to inactivation of the GTPase activity by preventing the interaction with downstream effectors, thereby inhibiting actin assembly in infected cells. It is unclear whether some human enzyme mediates AMPylation; FICD has such ability in vitro but additional experiments remain to

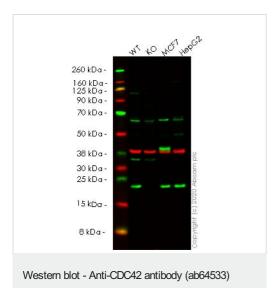
be done to confirm results in vivo.

Cellular localization Cell membrane.

Form There are 2 isoforms produced by alternative splicing. Isoform 1 also known as: Brain; Isoform 2

also known as: Placental.

Images



All lanes: Anti-CDC42 antibody (ab64533) at 1 µg/ml

Lane 1: Wild-type HEK-293T cell lysate

Lane 2: CDC42 knockout HEK-293T cell lysate

Lane 3 : MCF7 cell lysate
Lane 4 : HepG2 cell lysate

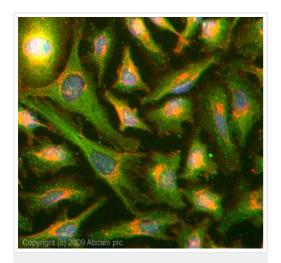
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 21 kDa

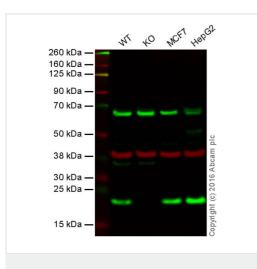
Lanes 1-4: Merged signal (red and green). Green - ab64533 observed at 20 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab64533 Anti-CDC42 antibody was shown to specifically react with CDC42 in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab266522 (knockout cell lysate ab256868) was used. Wild-type and CDC42 knockout samples were subjected to SDS-PAGE. ab64533 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 µg/ml and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-CDC42 antibody (ab64533)

ICC/IF image of ab64533 stained HeLa cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal Goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab64533, 5µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 Goat anti-Rabbit lgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue). This antibody also gave a positive result in 4% pfa fixed (10 min) HeLa cells at 5µg/ml.



Western blot - Anti-CDC42 antibody (ab64533)

All lanes: Anti-CDC42 antibody (ab64533) at 1 µg/ml

Lane 1: Wild-type HAP1 cell lysate

Lane 2: CDC42 knockout HAP1 cell lysate

Lane 3 : MCF7 cell lysate
Lane 4 : HepG2 cell lysate

Lysates/proteins at 20 µg per lane.

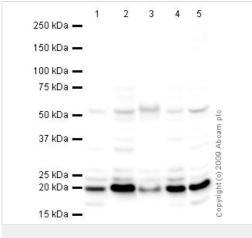
Predicted band size: 21 kDa

Lanes 1 - 4: Merged signal (red and green). Green – ab64533 observed at 20 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

Ab64533 was shown to recognize CDC42 in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when CDC42 knockout samples were eexamined. Wild-type and CDC42 knockout samples were subjected to SDS-PAGE.

Ab64533 and ab8245 (loading control to GAPDH) were diluted at 1µg/ml and 1/10,000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at

1/10,000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-CDC42 antibody (ab64533)

All lanes: Anti-CDC42 antibody (ab64533) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate

Lane 2 : Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate

Lane 3 : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate

Lane 4 : MCF7 (Human breast adenocarcinoma cell line) Whole Cell Lysate

Lane 5: U2OS (Human osteosarcoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat polyclonal to Rabbit lgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Developed using the ECL technique.

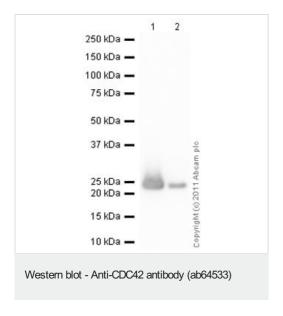
Performed under reducing conditions.

Predicted band size: 21 kDa **Observed band size:** 21 kDa

Additional bands at: 52 kDa. We are unsure as to the identity of

these extra bands.

Exposure time: 3 minutes



All lanes: Anti-CDC42 antibody (ab64533) at 1 µg/ml

Lane 1 : Recombinant Human CDC42 protein ($\underline{ab87713}$) at 0.1 µg **Lane 2 :** Recombinant Human CDC42 protein ($\underline{ab87713}$) at 0.01

μg

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 21 kDa

Exposure time: 1 minute

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