# abcam

### Product datasheet

# Anti-CDC42 antibody [EPR15620] ab187643





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#### Overview

**Product name** Anti-CDC42 antibody [EPR15620]

**Description** Rabbit monoclonal [EPR15620] to CDC42

**Host species** Rabbit

**Tested applications** Suitable for: IHC-P, IP, WB, Flow Cyt (Intra), ICC/IF

Species reactivity Reacts with: Mouse, Rat, Human

**Immunogen** Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HEK-293T, HepG2, MCF7, HT-29, Jurkat and HeLa whole cell lysate (ab150035); Mouse

> brain, Mouse kidney, Mouse spleen and Rat spleen lysates. IP: HT-29 cell lysate. Flow Cyt (intra): HeLa cells. ICC/IF: U937 cells. IHC-P: Human lung carcinoma, breast carcinoma and colon

tissues; Rat colon tissue.

**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** 

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

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

**Purity** Protein A purified

Clonality Monoclonal Clone number EPR15620

**Isotype** IgG

#### **Applications**

#### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab187643 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
IHC-P		1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/20 - 1/40.
WB		1/10000 - 1/50000. Detects a band of approximately 21 kDa (predicted molecular weight: 21 kDa).
Flow Cyt (Intra)		1/170.  ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ICC/IF	<b>★★★★</b> (1)	1/500.

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**Function** 

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, actinrich surface projections called filopodia.

Sequence similarities

Belongs to the small GTP as e superfamily. Rho family. CDC42 subfamily.

Post-translational modifications

AMPylation at Tyr-32 and Thr-35 are mediated by bacterial enzymes in case of infection by H.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** 

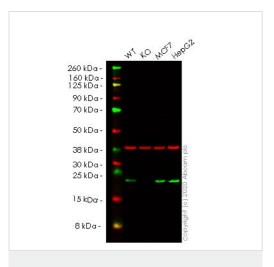
Cell membrane.

**Form** 

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

also known as: Placental.

#### **Images**



Western blot - Anti-CDC42 antibody [EPR15620] (ab187643)

**All lanes :** Anti-CDC42 antibody [EPR15620] (ab187643) at 1/5000 dilution

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

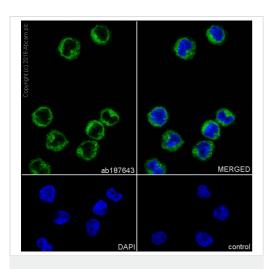
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 21 kDa

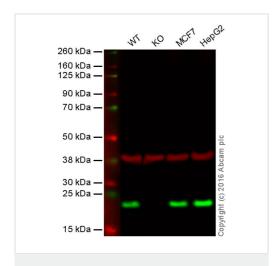
**Lanes 1-4:** Merged signal (red and green). Green - ab187643 observed at 20 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab187643 Anti-CDC42 antibody [EPR15620] was shown to specifically react with CDC42 in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line <a href="mailto:ab266522">ab266522</a> (knockout cell lysate <a href="mailto:ab256868">ab256868</a>) was used. Wild-type and CDC42 knockout samples were subjected to SDS-PAGE. ab187643 and Anti-GAPDH antibody [6C5] - Loading Control (<a href="mailto:ab8245">ab8245</a>) were incubated overnight at 4°C at 1 in 5000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<a href="mailto:ab216773">ab216773</a>) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (<a href="mailto:ab216776">ab216776</a>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-CDC42 antibody [EPR15620] (ab187643)

Immunocytochemistry/Immunofluorescence analysis of U937 (Human histiocytic lymphoma cell line) labelling CDC42 with purified ab187643 at 1/500. Cells were fixed with 4% PFA and permeabilized with 0.1% triton X-100. <a href="mailto:ab150077">ab150077</a> Goat anti rabbit lgG (Alexa Fluor<sup>®</sup> 488) at 1/1000 was used as the secondary antibody. Nuclei were counterstained with DAPI. PBS was used instead of the primary antibody as the negative control.



Western blot - Anti-CDC42 antibody [EPR15620] (ab187643)

**All lanes :** Anti-CDC42 antibody [EPR15620] (ab187643) at 1/5000 dilution

Lane 1: Wild-type HAP1 cell lysate

Lane 2: CDC42 knockout HAP1 cell lysate

Lane 3 : MCF7 cell lysate

Lane 4 : HepG2 cell lysate

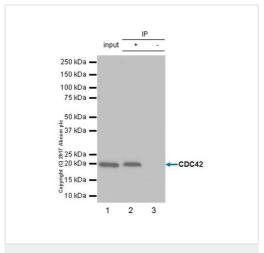
Lysates/proteins at 20 µg per lane.

Predicted band size: 21 kDa

**Lanes 1 - 4:** Merged signal (red and green). Green - ab187643 observed at 20 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

ab187643 was shown to specifically react with CDC42 in wild-type HAP1 cells. No band was observed when CDC42 knockout samples were examined. Wild-type and CDC42 knockout samples were subjected to SDS-PAGE. ab187643 and <a href="mailto:ab8245">ab8245</a> (loading control to GAPDH) were diluted at 1/5000 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.



Immunoprecipitation - Anti-CDC42 antibody [EPR15620] (ab187643)

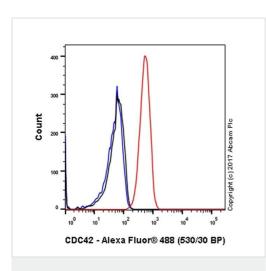
Lane 1 (input): HT-29 (human colorectal adenocarcinoma epithelial cell) whole cell lysate,10µg

Lane 2(+): HT-29 whole cell lysate

Lane 3(-): Rabbit monoclonal IgG (ab172730) instead of ab187643 in HT-29 whole cell lysate

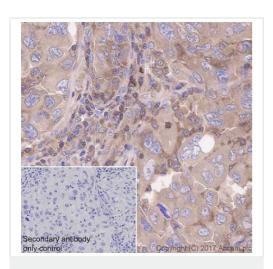
Ab187643 immunoprecipitating CDC42 in HT-29 whole cell lysate. Capture antibody was used at a 1:60 dilution. For western blotting, ab187643 was used as the primary antibody at a 1:1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST



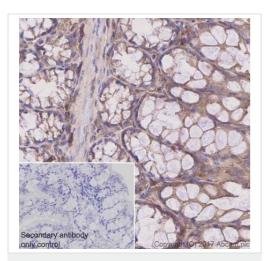
Flow Cytometry (Intracellular) - Anti-CDC42 antibody [EPR15620] (ab187643)

Intracellular Flow Cytometry analysis of HeLa cells (human cervix adenocarcinoma epithelial). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Primary antibody was used at a 1/120 dilution (red). A Goat anti rabbit lgG (Alexa Fluor<sup>®</sup>488, **ab150077**) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal lgG (**ab172730**) was used as the isotype control (black). Cell without incubation with primary antibody and secondary antibody (Blue).



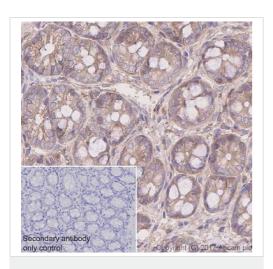
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CDC42 antibody
[EPR15620] (ab187643)

ab187643 staining CDC42 in Human lung carcinoma tissue sections by Immunohistochemistry (IHC-P- paraformaldehyde-fixed, paraffin-embedded sections). Antigen retrieval was by heat mediation using <a href="mailto:ab93684">ab93684</a> (Tris/EDTA buffer, pH 9.0). Samples were incubated with primary antibody at a 1/500 dilution. A ready to use Goat Anti-Rabbit IgG H&L (HRP) was used as the secondary antibody. Hematoxylin was used as a counter stain. Cytoplasmic staining on human lung carcinoma.



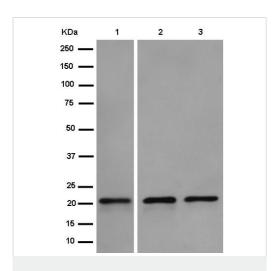
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CDC42 antibody
[EPR15620] (ab187643)

ab187643 staining CDC42 in Mouse colon tissue sections by Immunohistochemistry (IHC-P- paraformaldehyde-fixed, paraffinembedded sections). Antigen retrieval was by heat mediation using <a href="mailto:ab93684">ab93684</a> (Tris/EDTA buffer, pH 9.0). Samples were incubated with primary antibody at a 1/500 dilution. A ready to use Goat Anti-Rabbit IgG H&L (HRP) was used as the secondary antibody. Hematoxylin was used as a counter stain. Cytoplasmic staining on mouse colon.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CDC42 antibody
[EPR15620] (ab187643)

ab187643 staining CDC42 in Rat colon tissue sections by Immunohistochemistry (IHC-P- paraformaldehyde-fixed, paraffinembedded sections). Antigen retrieval was by heat mediation using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0). Samples were incubated with primary antibody at a 1/500 dilution. A ready to use Goat Anti-Rabbit IgG H&L (HRP) was used as the secondary antibody. Hematoxylin was used as a counter stain. Cytoplasmic staining on rat colon.



Western blot - Anti-CDC42 antibody [EPR15620] (ab187643)

**All lanes :** Anti-CDC42 antibody [EPR15620] (ab187643) at 1/20000 dilution

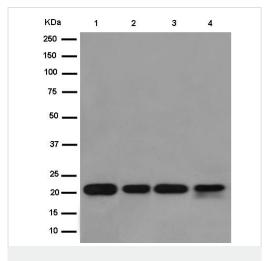
Lane 1 : Jurkat cell lysate
Lane 2 : HT-29 cell lysate
Lane 3 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

**All lanes :** Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugate at 1/1000 dilution

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



Western blot - Anti-CDC42 antibody [EPR15620] (ab187643)

**All lanes :** Anti-CDC42 antibody [EPR15620] (ab187643) at 1/5000 dilution

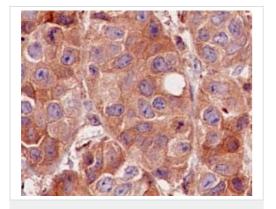
Lane 1 : Mouse brain lysate
Lane 2 : Mouse kidney lysate
Lane 3 : Mouse spleen lysate
Lane 4 : Rat spleen lysate

Lysates/proteins at 10 µg per lane.

## **Secondary**

**All lanes :** Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugate at 1/1000 dilution

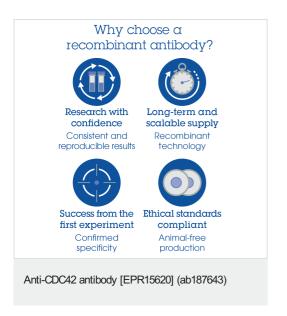
**Predicted band size:** 21 kDa **Observed band size:** 21 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CDC42 antibody
[EPR15620] (ab187643)

Immunohistochemical analysis of paraffin-embedded Human breast carcinoma tissue labeling CDC42 with ab187643 at 1/250 dilution, followed by prediluted HRP Polymer for Rabbit lgG. Counter stained with Hematoxylin.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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