abcam

Product datasheet

Anti-CDC42 antibody [EPR15620] - Low endotoxin, Azide free ab221781

KO VALIDATED Recombinant RabMAb

9 Images

Overview

Product name	Anti-CDC42 antibody [EPR15620] - Low endotoxin, Azide free
Description	Rabbit monoclonal [EPR15620] to CDC42 - Low endotoxin, Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, ICC/IF, IP, WB, Flow Cyt (Intra)
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, MCF7, HepG2, HT-29, Jurkat and HeLa cell lysates. Flow Cyt (intra): HeLa cells. IP: HT-29 cell lysate. ICC/IF: U937 cells. IHC-P: Rat and mouse colon tissues; Human lung and breast carcinoma tissues.
General notes	ab221781 is the carrier-free version of **ab187643**. 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**.

Our **Low endotoxin, azide-free formats** have low endotoxin level (≤ 1 EU/ml, determined by the LAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR15620
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab221781 in the following tested applications.

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

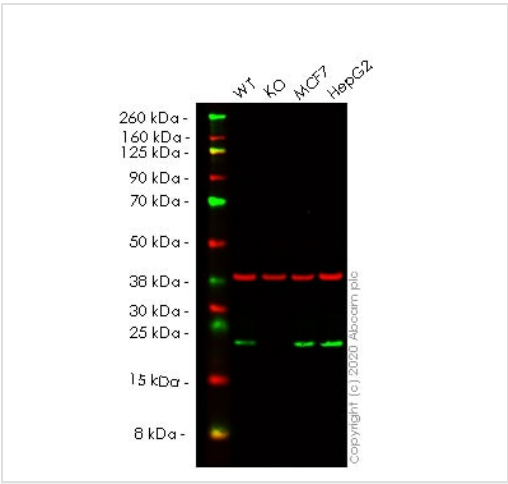
Application	Abreviews	Notes
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 21 kDa (predicted molecular weight: 21 kDa).
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target

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, actin-rich surface projections called filopodia.

Sequence similarities	Belongs to the small GTPase 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] - Low endotoxin, Azide free (ab221781)

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.

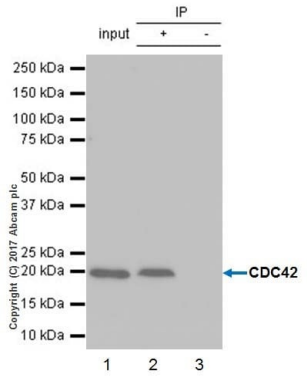
Predicted band size: 21 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab187643**).

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 **ab266522** (knockout cell lysate **ab256868**) was used. Wild-type and CDC42 knockout samples were subjected to SDS-PAGE. **ab187643** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 5000 dilution and 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.



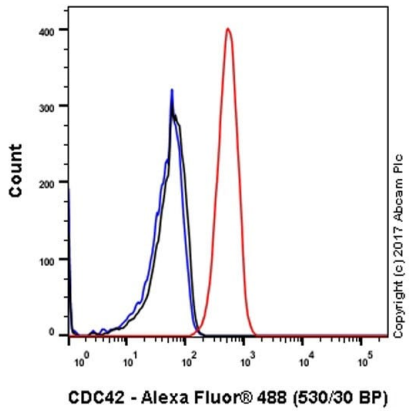
Immunoprecipitation - Anti-CDC42 antibody [EPR15620] - Low endotoxin, Azide free (ab221781)

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. Ab131366 Veriblot for IP (HRP) was used for detection at 1/1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST

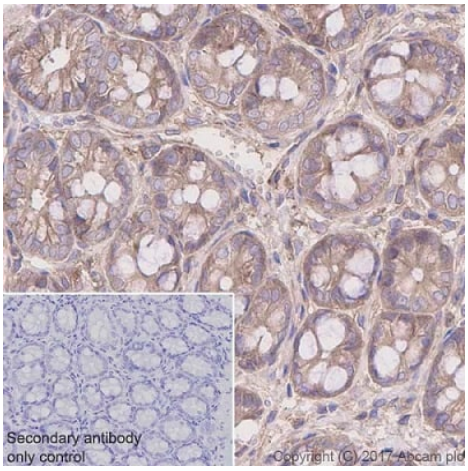
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab187643**).



Flow Cytometry (Intracellular) - Anti-CDC42 antibody [EPR15620] - Low endotoxin, Azide free (ab221781)

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 IgG (Alexa Fluor® 488, **ab150077**) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (**ab172730**) was used as the isotype control (black). Cell without incubation with primary antibody and secondary antibody (Blue).

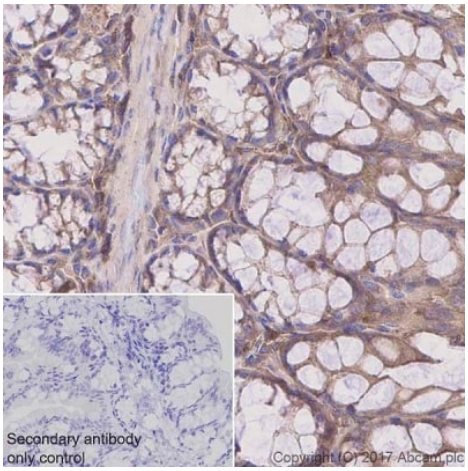
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab187643**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CDC42 antibody [EPR15620] - Low endotoxin, Azide free (ab221781)

ab187643 staining CDC42 in Rat colon tissue sections by Immunohistochemistry (IHC-P- paraformaldehyde-fixed, paraffin-embedded sections). Antigen retrieval was by heat mediation using **ab93684** (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.

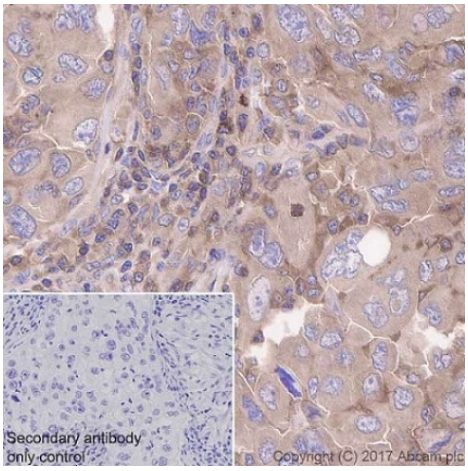
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab187643**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CDC42 antibody [EPR15620] - Low endotoxin, Azide free (ab221781)

ab187643 staining CDC42 in Mouse colon tissue sections by Immunohistochemistry (IHC-P- paraformaldehyde-fixed, paraffin-embedded sections). Antigen retrieval was by heat mediation using **ab93684** (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.

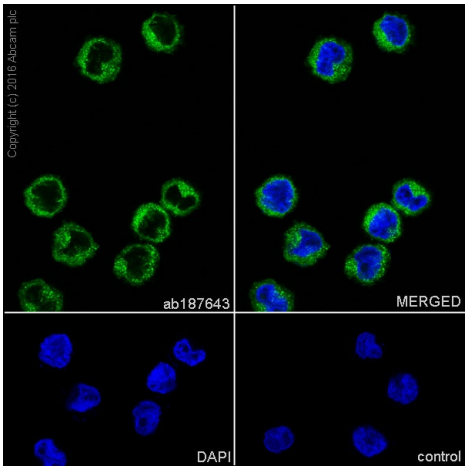
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab187643**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CDC42 antibody [EPR15620] - Low endotoxin, Azide free (ab221781)

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 **ab93684** (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.

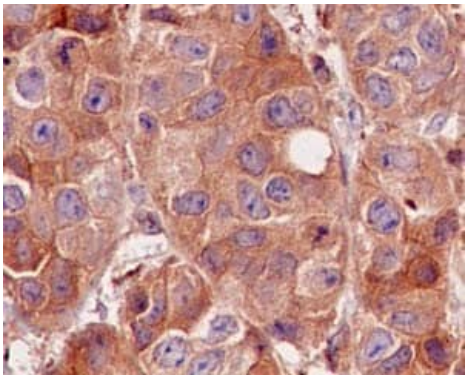
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab187643**).



Immunocytochemistry/ Immunofluorescence - Anti-CDC42 antibody [EPR15620] - Low endotoxin, Azide free (ab221781)

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. **ab150077** Goat anti rabbit IgG (Alexa Fluor® 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab187643**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CDC42 antibody [EPR15620] - Low endotoxin, Azide free (ab221781)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab187643**).

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

Anti-CDC42 antibody [EPR15620] - Low endotoxin, Azide free (ab221781)

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