abcam

Product datasheet

Anti-RhoA antibody [EPR18134] - Low endotoxin, Azide free ab219371

KO VALIDATED Recombinant RabMAb

2 References 6 Images

Overview

Product name	Anti-RhoA antibody [EPR18134] - Low endotoxin, Azide free
Description	Rabbit monoclonal [EPR18134] to RhoA - Low endotoxin, Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human RhoA full length protein; HeLa, HEK293 C6, Raw264.7 and NIH 3T3 cell lysates, human fetal brain and fetal kidney lysates, mouse brain, kidney and spleen lysates, rat brain, kidney and spleen lysates. ICC/IF: Jurkat and K562 cells. Flow Cyt (intra): HeLa cells.
General notes	ab219371 is the carrier-free version of **ab187027**. 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	EPR18134
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab219371 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
WB		Use at an assay dependent concentration. Detects a band of approximately 22 kDa (predicted molecular weight: 22 kDa).
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

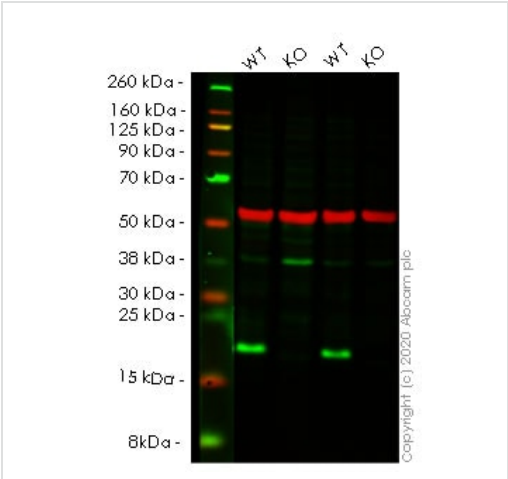
Target

Function	Regulates a signal transduction pathway linking plasma membrane receptors to the assembly of focal adhesions and actin stress fibers. Serves as a target for the yopT cysteine peptidase from Yersinia pestis, vector of the plague, and Yersinia pseudotuberculosis, which causes gastrointestinal disorders. May be an activator of PLCE1. Activated by ARHGEF2, which promotes the exchange of GDP for GTP.
Sequence similarities	Belongs to the small GTPase superfamily. Rho family.
Domain	The basic-rich region is essential for yopT recognition and cleavage.
Post-translational modifications	Substrate for botulinum ADP-ribosyltransferase. Cleaved by yopT protease when the cell is infected by some Yersinia pathogens. This removes

the lipid attachment, and leads to its displacement from plasma membrane and to subsequent cytoskeleton cleavage.
AMPylation at Tyr-34 and Thr-37 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.
Ubiquitinated by the BCR(BACURD1) and BCR(BACURD2) E3 ubiquitin ligase complexes, leading to its degradation by the proteasome, thereby regulating the actin cytoskeleton and cell migration.

Cellular localization Cell membrane. Cytoplasm > cytoskeleton.

Images



Western blot - Anti-RhoA antibody [EPR18134] - Low endotoxin, Azide free (ab219371)

All lanes : Anti-RhoA antibody [EPR18134] (**ab187027**) at 1/5000 dilution

Lanes 1 & 3 : Wild-type HEK-293T cell lysate
Lanes 2 & 4 : RHOA knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

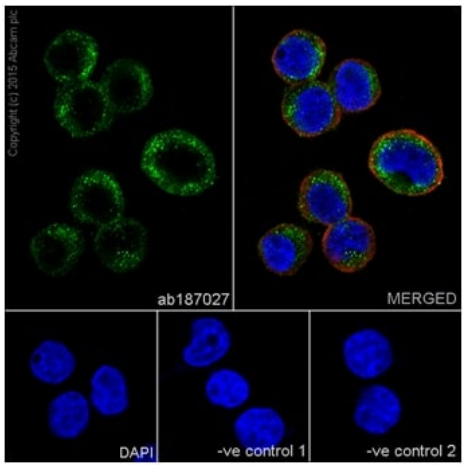
Predicted band size: 22 kDa
Observed band size: 21 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab187027**).

Lanes 1- 4: Merged signal (red and green). Green - **ab187027** observed at 21 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

ab187027 was shown to react with RhoA in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab266592** (knockout cell lysate **ab257637**) was used. Wild-type HEK-293T and RHOA knockout HEK-293T 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. **ab187027** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) overnight at 4°C at a 1 in 5000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-

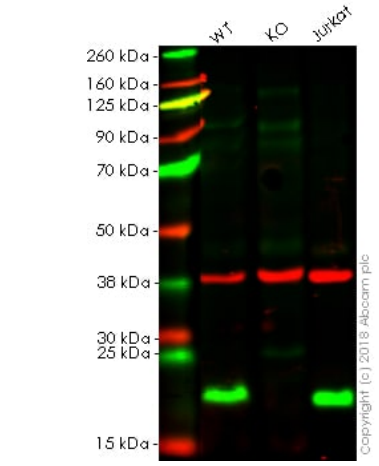
Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-RhoA antibody [EPR18134] - Low endotoxin, Azide free (ab219371)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Jurkat (Human T cell leukemia cells from peripheral blood) cells labeling RhoA with **ab187027** at 1/150 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on Jurkat cell line is observed. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).
The negative controls are as follows:
1. **ab187027** at 1/150 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution.
2. **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab187027**).



Western blot - Anti-RhoA antibody [EPR18134] - Low endotoxin, Azide free (ab219371)

All lanes : Anti-RhoA antibody [EPR18134] (**ab187027**) at 1/1000 dilution

Lane 1 : Wild-type Hek293T whole cell lysate
Lane 2 : RHOA knockout Hek293T whole cell lysate
Lane 3 : Jurkat whole cell lysate

Lysates/proteins at 20 µg per lane.

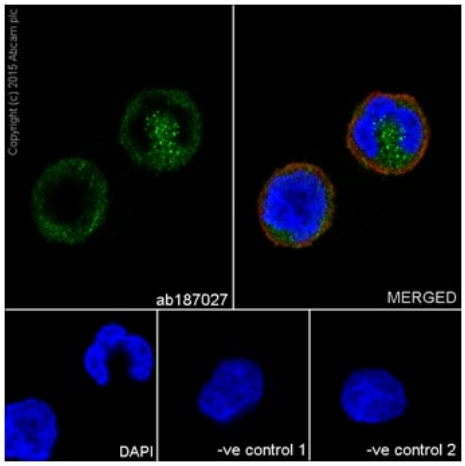
Predicted band size: 22 kDa

Lanes 1 - 3: Merged signal (red and green). Green - **ab187027** observed at 22 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab187027 was shown to specifically react with RhoA in wild-type Hek293T cells as signal was lost in RHOA knockout cells. Wild-type

and RHOA knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% Milk. Ab187027 and **ab9484** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

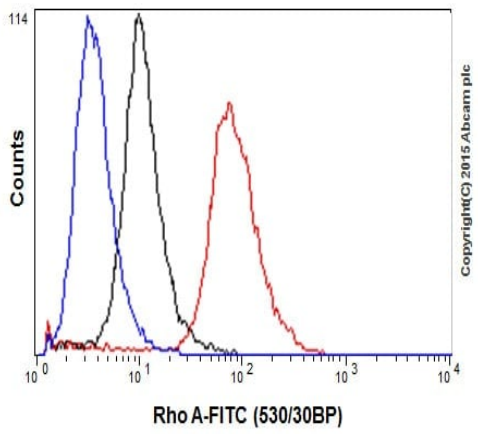
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab187027**).



Immunocytochemistry/ Immunofluorescence - Anti-RhoA antibody [EPR18134] - Low endotoxin, Azide free (ab219371)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized K562 (Human chronic myelogenous leukemia cells from bone marrow) cells labeling RhoA with **ab187027** at 1/150 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on K562 cell line is observed. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).
The negative controls are as follows:
1. **ab187027** at 1/150 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution.
2. **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.

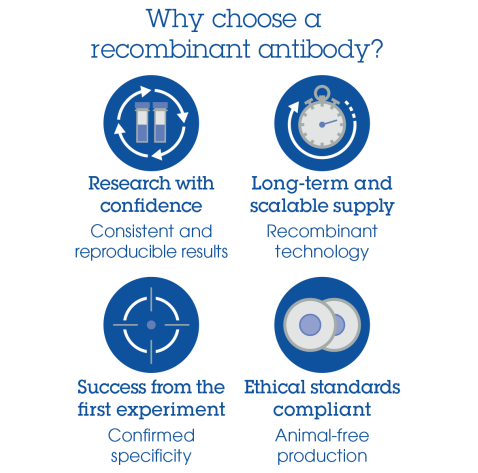
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab187027**).



Flow Cytometry (Intracellular) - Anti-RhoA antibody [EPR18134] - Low endotoxin, Azide free (ab219371)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling RhoA with **ab187027** at 1/200 dilution (red) compared with a rabbit monoclonal IgG isotype control (**ab172730**; black) and a unlabeled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/500 dilution 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 (**ab187027**).



Anti-RhoA antibody [EPR18134] - Low endotoxin, Azide free (ab219371)

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