abcam

ab219371

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

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR18134
Purification technique	Affinity purification Protein A
Concentration	1.017 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human, Mouse, Rat
Dilution info	-

Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information RHOA

Function	Small GTPase which cycles between an active GTP-bound and an inactive GDP-bound state. Mainly associated with cytoskeleton organization, in active state binds to a variety of effector proteins to regulate cellular responses such as cytoskeletal dynamics, cell migration and cell cycle (PubMed:23871831). Regulates

a signal transduction pathway linking plasma membrane receptors to the assembly of focal adhesions and actin stress fibers (PubMed:31570889, PubMed:8910519, PubMed:9121475). Involved in a microtubule-dependent signal that is required for the myosin contractile ring formation during cell cycle cytokinesis (PubMed:12900402, PubMed:16236794). Plays an essential role in cleavage furrow formation. Required for the apical junction formation of keratinocyte cell-cell adhesion (PubMed:20974804, PubMed:23940119). Essential for the SPATA13-mediated regulation of cell migration and adhesion assembly and disassembly (PubMed:19934221). The MEMO1-RHOA-DIAPH1 signaling pathway plays an important role in ERBB2-dependent stabilization of microtubules at the cell cortex. It controls the localization of APC and CLASP2 to the cell membrane, via the regulation of GSK3B activity. In turn, membrane-bound APC allows the localization of the MACF1 to the cell membrane, which is required for microtubule capture and stabilization (PubMed:20937854). Involved in the reorientation of endothelial cells and their actin stress fibers in response to cellular mechantransduction-mediated activation by ARHGEF40 (By similarity). Regulates KCNA2 potassium channel activity by reducing its location at the cell surface in response to CHRM1 activation; promotes KCNA2 endocytosis (PubMed:19403695, PubMed:9635436). Acts as an allosteric activator of guanine nucleotide exchange factor ECT2 by binding in its activated GTP-bound form to the PH domain of ECT2 which stimulates the release of PH inhibition and promotes the binding of substrate RHOA to the ECT2 catalytic center (PubMed:31888991). May be an activator of PLCE1 (PubMed:16103226). In neurons, involved in the inhibition of the initial spine growth. Upon activation by CaMKII, modulates dendritic spine structural plasticity by relaying CaMKII transient activation to synapse-specific, long-term signaling (By similarity). Acts as a regulator of platelet alpha-granule release during activation and aggregation of platelets (By similarity). When activated by DAAM1 may signal centrosome maturation and chromosomal segregation during cell division. May also be involved in contractile ring formation during cytokinesis. (Microbial infection) Serves as a target for the yopT cysteine peptidase from Yersinia pestis, vector of the plague.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab219371 is the carrier-free version of ab187027.

Patented technology

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility
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.

What does low endotoxin mean?
Our low endotoxin, azide-free formats have low endotoxin level (1 EU/mg, determined by the TAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	RhoA also known as Ras homolog family member A is a small signaling GTPase. It weighs approximately 21 kDa. This protein is a part of the Rho family which includes other members like RhoB and RhoC. RhoA is widely expressed in various tissues including the brain kidney liver and lungs. Mechanically RhoA functions as a molecular switch cycling between an active GTP-bound state and an inactive GDP-bound state. This activity regulates the organization of the actin cytoskeleton and influences cell shape attachment and motility.
Biological function summary	The role of RhoA extends to cell proliferation and differentiation. It is a component of the Rho-GTPase cycle interacting with numerous effectors to transmit signals from the cell membrane to the actin cytoskeleton. RhoA influences the assembly of stress fibers and focal adhesions. It plays a part in cellular adhesion and migration making it essential for development and wound healing processes. As a central player in these activities RhoA affects several cellular responses to external stimuli.
Pathways	Research places RhoA prominently in the regulation of the Rho-ROCK (Rho-associated protein kinase) pathway and the planar cell polarity pathway. The Rho-ROCK pathway contributes to cytoskeletal dynamics and cell contractility while the planar cell polarity pathway influences tissue architecture during embryonic development. RhoA interacts with G17A in these pathways coordinating activities such as smooth muscle contraction and neuronal growth. These interactions also involve cross-talk with other signals linked to GTPases like Rac1 and Cdc42.

Associated diseases and disorders	RhoA has connections to cancer progression and hypertension. In cancer RhoA regulates actin dynamics influencing tumor cell migration and invasion. It forms interactions with proteins such as p53 affecting apoptotic pathways and tumor suppression. In hypertension RhoA's role in smooth muscle contraction affects blood vessel tension and blood pressure regulation. Abnormal activity in the Rho-ROCK pathway contributes to these disorders highlighting the significance of RhoA in disease mechanisms.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

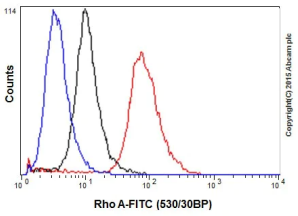
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

5 product images

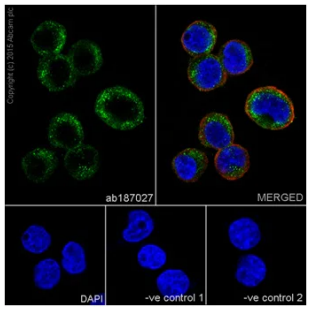


Flow Cytometry (Intracellular) - Anti-RhoA antibody [EPR18134] - Low endotoxin, Azide free (ab219371)

RhoA Flow Cytometry (Intracellular) staining using rabbit Anti-RhoA antibody

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.

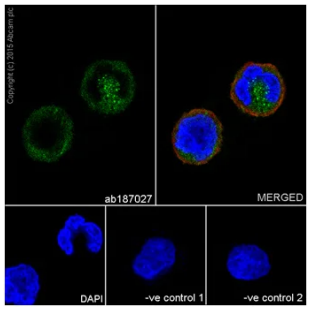
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab187027).



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

RhoA Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-RhoA antibody

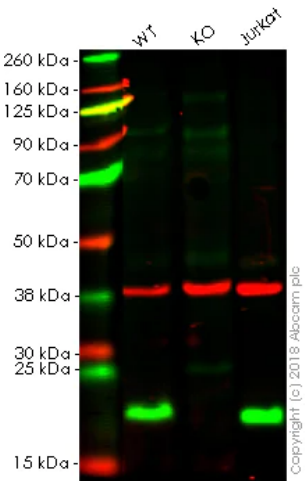
Immunofluorescent analysis of 4% paraformaldehyde-fixed 0.1% Triton X-100 permeabilized Jurkat (Human T cell leukemia cells from peripheral blood) cells labeling RhoA with ab187027 at 1/150 dilution followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). 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).



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

RhoA Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-RhoA antibody

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).



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

RhoA Western blot staining using rabbit Anti-RhoA antibody

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).

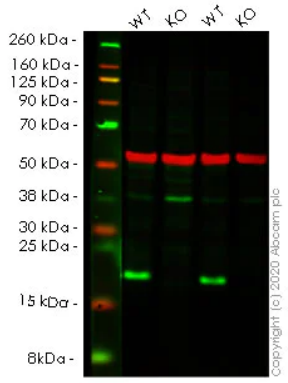
All lanes:
Western blot - Anti-RhoA antibody [EPR18134] (ab187027) at 1/1000 dilution

Lane 1:
Wild-type Hek293T whole cell lysate at 20 µg

Lane 2:
RHOA knockout Hek293T whole cell lysate at 20 µg

Lane 3:
Jurkat whole cell lysate at 20 µg

Predicted band size: 22 kDa



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

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.

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

Lanes 1 and 3:
Wild-type HEK-293T cell lysate at 20 µg

Lanes 2 and 4:
RHOA knockout HEK-293T cell lysate at 20 µg

Lane 2:
Western blot - Human RHOA knockout HEK-293T cell line (ab266592)

Performed under reducing conditions.

Predicted band size: 22 kDa

Observed band size: 21 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.