abcam

Product datasheet

Anti-RhoA antibody [EPR18134] ab187027

KO VALIDATED Recombinant RabMAb

1 Abreviews 80 References 10 Images

Overview

Product name	Anti-RhoA antibody [EPR18134]
Description	Rabbit monoclonal [EPR18134] to RhoA
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, ICC/IF
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, HEK-293 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	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	Liquid
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: PBS, 0.05% BSA, 40% Glycerol
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR18134

Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab187027 in the following tested applications.

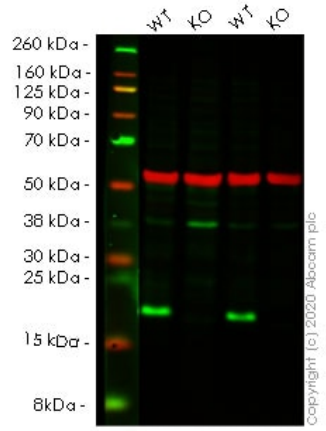
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/200.
WB	★★★★☆ **(1)**	1/5000. Detects a band of approximately 22 kDa (predicted molecular weight: 22 kDa).
ICC/IF		1/150.

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] (ab187027)

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

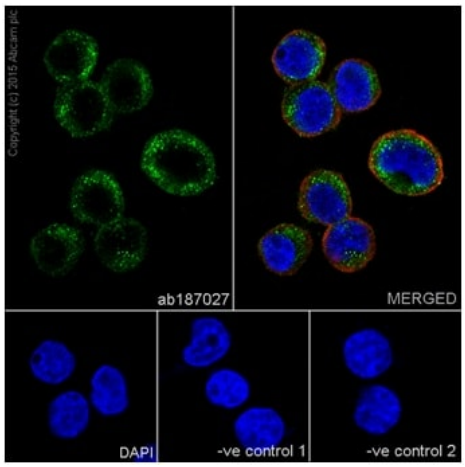
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

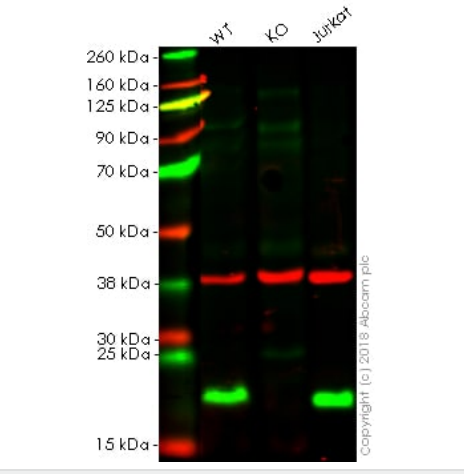
Lanes 1- 4: Merged signal (red and green). Green - ab187027 observed at 21 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

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] (ab187027)

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.



Western blot - Anti-RhoA antibody [EPR18134] (ab187027)

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

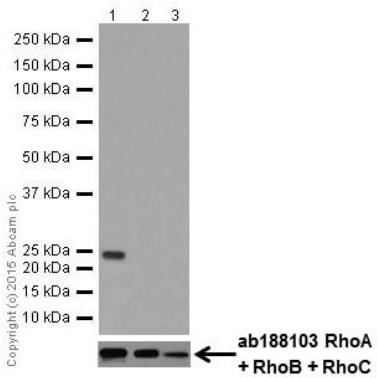
Lysates/proteins at 20 µg per lane.

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.

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.



Western blot - Anti-RhoA antibody [EPR18134] (ab187027)

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

Lane 1 : Human RhoA full length protein
Lane 2 : Human RhoB full length protein
Lane 3 : Human RhoC full length protein

Lysates/proteins at 0.01 µg per lane.

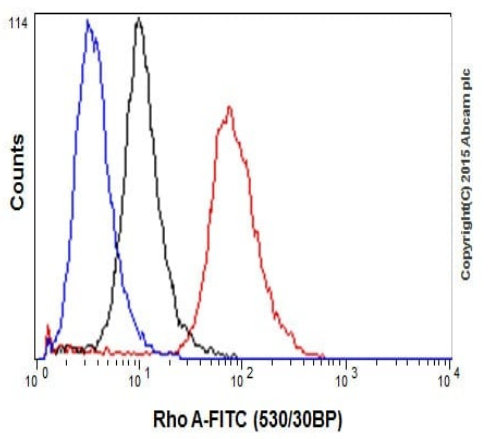
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/1000 dilution

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

Exposure time: 1 second

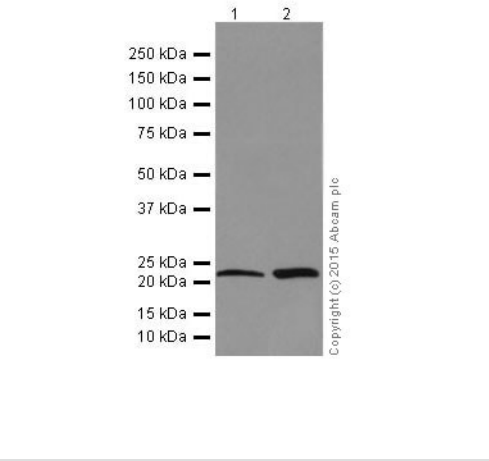
5% NFDM/TBST: Blocking and diluting buffer.

Human RhoA full length protein (**ab101594**) contains aa1-193 with His-Tag® at N-Terminus; Human RhoB full length protein (**ab107139**) contains aa1-193 with His-Tag® at N-Terminus; Human RhoC full length protein (**ab98085**) contains aa1-190 with His-Tag® at N-Terminus.



Flow Cytometry (Intracellular) - Anti-RhoA antibody [EPR18134] (ab187027)

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.



Western blot - Anti-RhoA antibody [EPR18134] (ab187027)

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

Lane 1 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate
Lane 2 : HEK-293 (Human epithelial cells from embryonic kidney) whole cell lysate

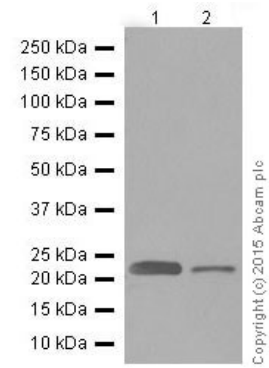
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/1000 dilution

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

Exposure time: 3 minutes

5% NFDM/TBST: Blocking and diluting buffer.



Western blot - Anti-RhoA antibody [EPR18134] (ab187027)

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

Lane 1 : Human fetal brain lysate
Lane 2 : Human fetal kidney lysate

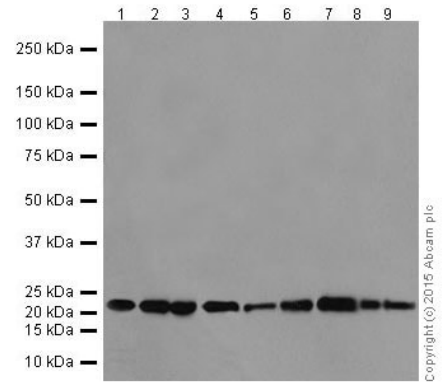
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

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

Exposure time: 3 minutes

5% NFDM/TBST: Blocking and diluting buffer.



Western blot - Anti-RhoA antibody [EPR18134] (ab187027)

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

Lane 1 : Mouse brain lysate
Lane 2 : Mouse kidney lysate
Lane 3 : Mouse spleen lysate
Lane 4 : Rat brain lysate
Lane 5 : Rat kidney lysate
Lane 6 : Rat spleen lysate
Lane 7 : C6 (Rat glial tumor cells) whole cell lysate
Lane 8 : Raw264.7 (Mouse macrophage cells transformed with Abelson murine leukemia virus) whole cell lysate
Lane 9 : NIH/3T3 (Mouse embryo fibroblast cells) whole cell lysate

Lysates/proteins at 10 µg per lane.

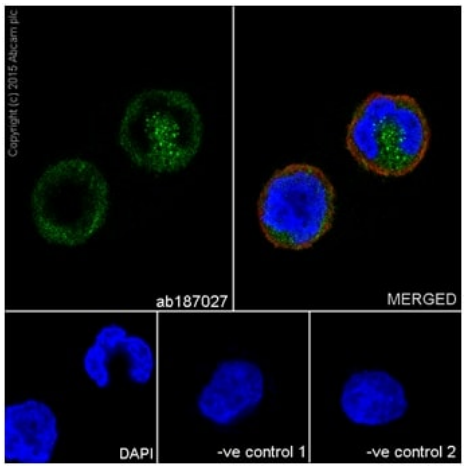
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/1000 dilution

Predicted band size: 22 kDa

Observed band size: 22 kDa

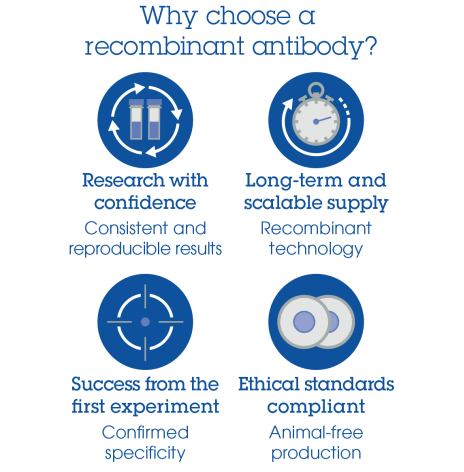
Exposure time: 3 minutes

5% NFDM/TBST: Blocking and diluting buffer.



Immunocytochemistry/ Immunofluorescence - Anti-RhoA antibody [EPR18134] (ab187027)

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.



Anti-RhoA antibody [EPR18134] (ab187027)

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours

- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors