abcam

Product datasheet

Anti-RhoA antibody [EPR18134] ab187027



ייבע RabMAb

★★★★ 1 Abreviews 80 References 画像数 10

製品の概要

製品名 Anti-RhoA antibody [EPR18134]

製品の詳細 Rabbit monoclonal [EPR18134] to RhoA

由来種 Rabbit

アプリケーション 適用あり: Flow Cyt (Intra), WB, ICC/IF

種交差性 交差種: Mouse, Rat, Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール 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.

特記事項 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**.

製品の特性

製品の状態 Liquid

保存方法 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.

バッファー pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 0.05% BSA, 40% Glycerol

精製度 Protein A purified

ポリ/モノ モノクローナル クローン名 EPR18134

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab187027の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
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.

ターゲット情報

機能 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.

配列類似性 Belongs to the small GTPase superfamily. Rho family.

ドメイン The basic-rich region is essential for yopT recognition and cleavage.

翻訳後修飾 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

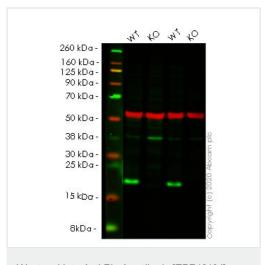
be done to confirm results in vivo.

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.

細胞内局在 Cell membrane. Cytoplasm > cytoskeleton.

画像



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

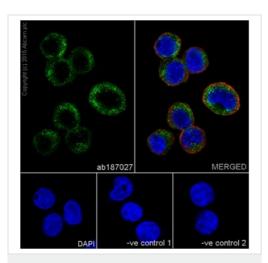
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

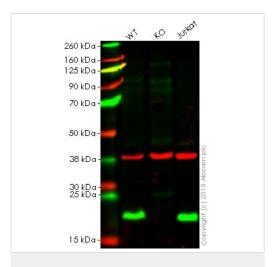
Predicted band size: 22 kDa **Observed band size:** 21 kDa

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



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



Western blot - 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 lgG (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. <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor[®]488 Goat Anti-Rabbit lgG H&L) at 1/1000 dilution.

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 <u>ab9484</u> (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 <u>ab216773</u> and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed <u>ab216776</u> secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

1 2 3

250 kDa —

150 kDa —

100 kDa —

75 kDa —

37 kDa —

37 kDa —

37 kDa —

48 25 kDa —

91 20 kDa —

115 kDa —

116 kDa —

116 kDa —

117 kDa —

48 25 kDa —

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

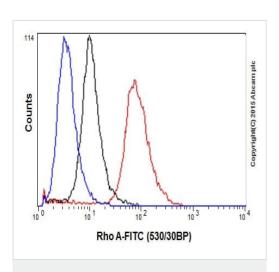
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/1000 dilution

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

Exposure time: 1 second

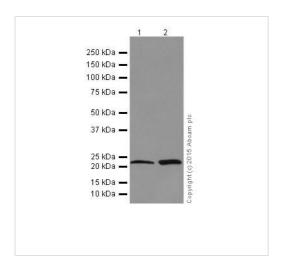
5% NFDM/TBST: Blocking and diluting buffer.

Human RhoA full length protein (<u>ab101594</u>) contains aa1-193 with His-Tag[®] at N-Terminus; Human RhoB full length protein (<u>ab107139</u>) contains aa1-193 with His-Tag[®] at N-Terminus; Human RhoC full length protein (<u>ab98085</u>) 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 lgG isotype control (ab172730; black) and a unlabeled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit lgG (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.

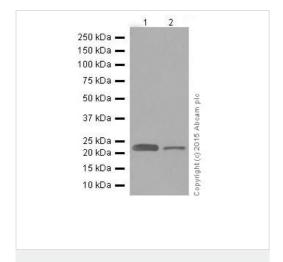
Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) 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)



Lane 1 : Human fetal brain lysate

Lane 2 : Human fetal kidney lysate

Lysates/proteins at 10 µg per lane.

Secondary

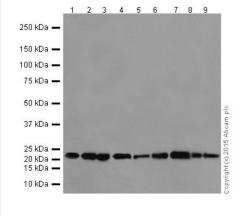
All lanes : Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG at 1/1000 dilution

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

Observed band size: 22 kD

Exposure time: 3 minutes

1 2 3 4 5 6 7 8 9



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

5% NFDM/TBST: Blocking and diluting buffer.

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) (<u>ab97051</u>) at 1/1000 dilution

Predicted band size: 22 kDa

Observed band size: 22 kDa

Exposure time: 3 minutes

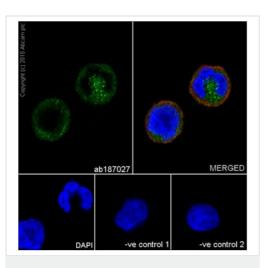
5% NFDM/TBST: Blocking and diluting buffer.

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 lgG (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).

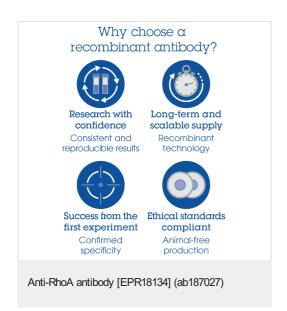
The negative controls are as follows:

1. ab187027 at 1/150 dilution followed by <u>ab150120</u> (AlexaFluor[®]594 Goat anti-Mouse secondary) at 1/1000 dilution.

2. <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor[®]488 Goat Anti-Rabbit lgG H&L) at 1/1000 dilution.



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



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