abcam

Product datasheet

Anti-ROCK1 antibody [EPR638Y] ab134181

敲除验证 重组 RabMAb

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概述

产品名称	Anti-ROCK1抗体[EPR638Y]
描述	兔单克隆抗体[EPR638Y] to ROCK1
宿主	Rabbit
特异性	The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
经测试应用	**适用于:** Flow Cyt (Intra), WB, IP, IHC-P
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: HeLa, HAP1, and HEK293 cell lysates, and Mouse brain and Rat brain tissue lysates IHC: Human testis tissue and Human breast carcinoma tissue IP: HeLa cell lysate Flow Cyt (Intra): HeLa cells
常规说明	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**.

性能

形式	Liquid
存放说明	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.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified

克隆	单克隆
克隆编号	EPR638Y
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab134181于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/150. Not suitable for unpurified form.
WB	★★★★☆ (1)	1/500 - 1/1000. Detects a band of approximately 160 kDa (predicted molecular weight: 158 kDa).
IP		1/60 - 1/120.
IHC-P		1/1200. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols** **For unpurified use at 1/50 - 1/100.** The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.

靶标

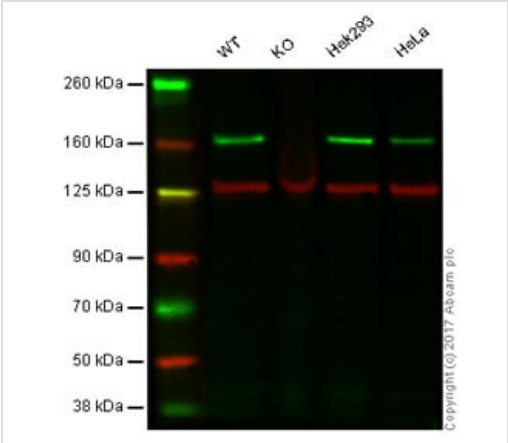
功能 Protein kinase which is a key regulator of actin cytoskeleton and cell polarity. Involved in regulation of smooth muscle contraction, actin cytoskeleton organization, stress fiber and focal adhesion formation, neurite retraction, cell adhesion and motility via phosphorylation of DAPK3, GFAP, LIMK1, LIMK2, MYL9/MLC2, PFN1 and PPP1R12A. Phosphorylates FHOD1 and acts synergistically with it to promote SRC-dependent non-apoptotic plasma membrane blebbing. Phosphorylates JIP3 and regulates the recruitment of JNK to JIP3 upon UVB-induced stress. Acts as a suppressor of inflammatory cell migration by regulating PTEN phosphorylation and stability. Acts as a negative regulator of VEGF-induced angiogenic endothelial cell activation. Required for centrosome positioning and centrosome-dependent exit from mitosis. Plays a role in terminal erythroid differentiation. May regulate closure of the eyelids and ventral body wall by inducing the assembly of actomyosin bundles. Promotes keratinocyte terminal differentiation. Involved in osteoblast compaction through the fibronectin fibrillogenesis cell-mediated matrix assembly process, essential for osteoblast mineralization.

组织特异性 Detected in blood platelets.

序列相似性 Belongs to the protein kinase superfamily. AGC Ser/Thr protein kinase family.
Contains 1 AGC-kinase C-terminal domain.
Contains 1 PH domain.
Contains 1 phorbol-ester/DAG-type zinc finger.
Contains 1 protein kinase domain.
Contains 1 REM (Hr1) repeat.

结构域	The C-terminal auto-inhibitory domain interferes with kinase activity. RHOA binding leads to a conformation change and activation of the kinase. Truncated ROCK1 is constitutively activated.
翻译后修饰	Autophosphorylated on serine and threonine residues. Cleaved by caspase-3 during apoptosis. This leads to constitutive activation of the kinase and membrane blebbing.
细胞定位	Cytoplasm. Cytoplasm, cytoskeleton, microtubule organizing center, centrosome, centriole. Golgi apparatus membrane. Cell projection, bleb. Cytoplasm, cytoskeleton. Cell membrane. Cell projection, lamellipodium. Cell projection, ruffle. Associated with the mother centriole and an intercentriolar linker. Colocalizes with ITGB1BP1 and ITGB1 at the cell membrane predominantly in lamellipodia and membrane ruffles, but also in retraction fibers. Localizes at the cell membrane in an ITGB1BP1-dependent manner (By similarity). A small proportion is associated with Golgi membranes.

图片

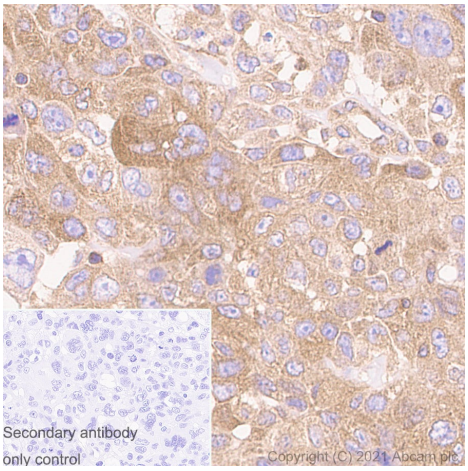


Western blot - Anti-ROCK1 antibody [EPR638Y] (ab134181)

Lane 1: Wild type HAP1 whole cell lysate (40 µg)
Lane 2: ROCK1 knockout HAP1 whole cell lysate (40 µg)
Lane 3: HEK293 whole cell lysate (20 µg)
Lane 4: HeLa whole cell lysate (20 µg)

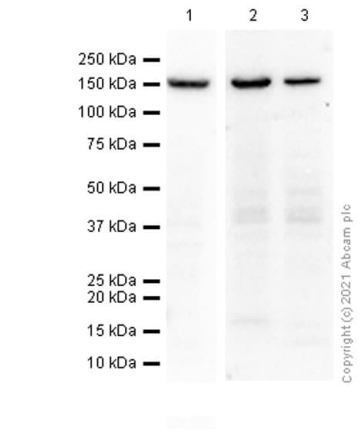
Lanes 1 - 4: Merged signal (red and green). Green - ab134181 observed at 165 kDa. Red - loading control, **ab18058**, observed at 130 kDa.

ab134181 was shown to specifically react with ROCK1 when ROCK1 knockout samples were used. Wild-type and ROCK1 knockout samples were subjected to SDS-PAGE. Ab134181 and **ab18058** (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 500 dilution and 1/10000 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 1/10000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ROCK1 antibody [EPR638Y] (ab134181)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue sections labeling ROCK1 with Purified ab134181 at 1:1200 (1.28 µg/ml). Heat mediated antigen retrieval was performed using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Western blot - Anti-ROCK1 antibody [EPR638Y] (ab134181)

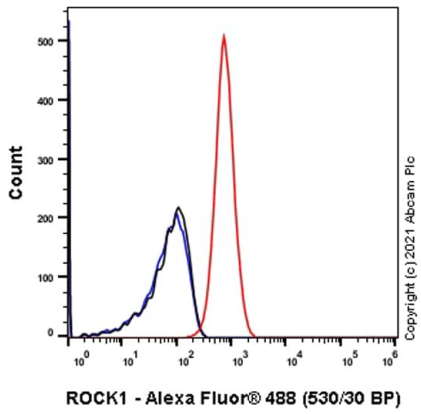
All lanes : Anti-ROCK1 antibody [EPR638Y] (ab134181) at 1/1000 dilution (Purified)

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : Mouse brain
Lane 3 : Rat brain

Lysates/proteins at 20 µg per lane.

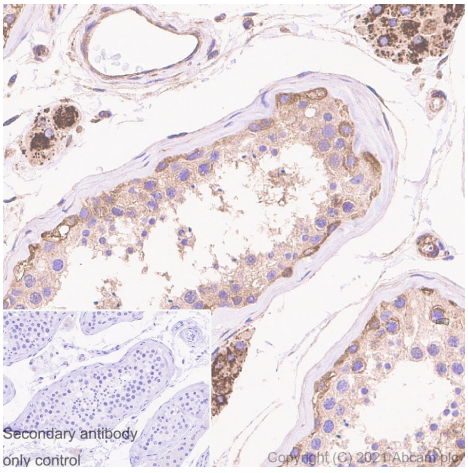
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 158 kDa
Observed band size: 158 kDa



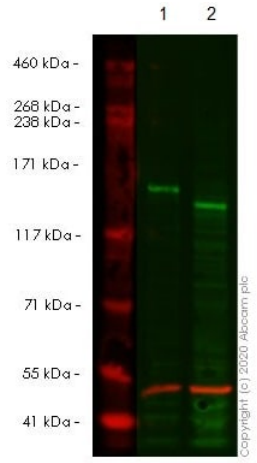
Flow Cytometry (Intracellular) - Anti-ROCK1 antibody [EPR638Y] (ab134181)

Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling ROCK1 with purified ab134181 at 1/150 dilution (10 µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150081**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (blue).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ROCK1 antibody [EPR638Y] (ab134181)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human testis tissue sections labeling ROCK1 with Purified ab134181 at 1:1200 (1.28 µg/ml). Heat mediated antigen retrieval was performed using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Western blot - Anti-ROCK1 antibody [EPR638Y] (ab134181)

All lanes : Anti-ROCK1 antibody [EPR638Y] (ab134181) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ROCK1 CRISPR/Cas9 edited HeLa cell lysate

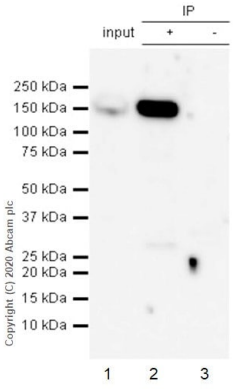
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 158 kDa
Observed band size: 160 kDa

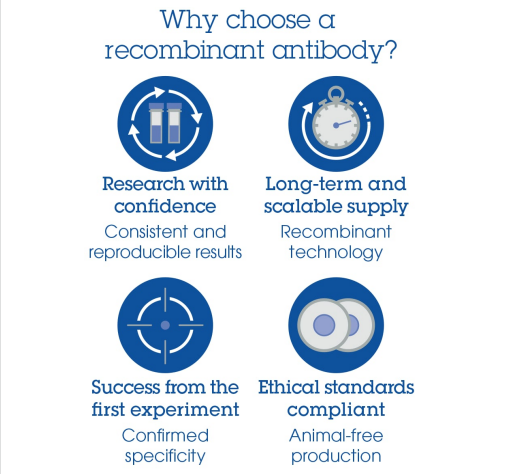
Lanes 1- 2: Merged signal (red and green). Green - ab134181 observed at 160 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

ab134181 was shown to react with ROCK1 in wild-type HeLa cells in western blot. The band observed in CRISPR/Cas9 edited cell line **ab264780** (CRISPR/Cas9 edited cell lysate **ab257642**) lane below 160kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HeLa and ROCK1 CRISPR/Cas9 edited HeLa 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. ab134181 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) were incubated overnight at 4°C at a 1 in 1000 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.



Immunoprecipitation - Anti-ROCK1 antibody [EPR638Y] (ab134181)

Purified ab134181 at 1/120 dilution (2µg) immunoprecipitating ROCK1 in HeLa whole cell lysate.
Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10µg
Lane 2 (+): ab134181 + HeLa whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab134181 in HeLa whole cell lysate.
VeriBlot for IP Detection Reagent (HRP) (**ab131366**) (1/1000 dilution) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 158 kDa



Anti-ROCK1 antibody [EPR638Y] (ab134181)

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