abcam

Product datasheet

Anti-ROCK1 antibody [EPR638Y] - BSA and Azide free ab230799

KO VALIDATED Recombinant RabMAb

8 Images

Overview

Product name	Anti-ROCK1 antibody [EPR638Y] - BSA and Azide free
Description	Rabbit monoclonal [EPR638Y] to ROCK1 - BSA and Azide free
Host species	Rabbit
Specificity	The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
Tested applications	**Suitable for:** WB, IP, IHC-P, 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: 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
General notes	ab230799 is the carrier-free version of **ab134181**. 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.

Properties

Form	Liquid

Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: 100% PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR638Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab230799 in the following tested applications.

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

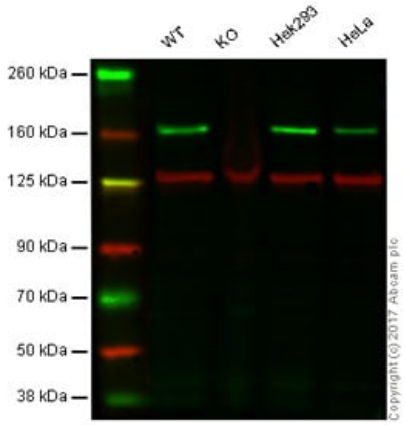
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 160 kDa (predicted molecular weight: 158 kDa).
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
Flow Cyt (Intra)		Use at an assay dependent concentration.

Target

Function	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.
Tissue specificity	Detected in blood platelets.
Sequence similarities	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.
Domain	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.
Post-translational modifications	Autophosphorylated on serine and threonine residues. Cleaved by caspase-3 during apoptosis. This leads to constitutive activation of the kinase and membrane blebbing.
Cellular localization	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.

Images



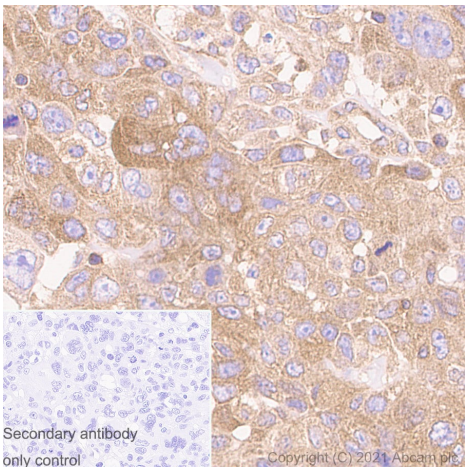
Western blot - Anti-ROCK1 antibody [EPR638Y] - BSA and Azide free (ab230799)

This WB data was generated using the same anti-ROCK1 antibody clone, EPR638Y, in a different buffer formulation (cat# **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)

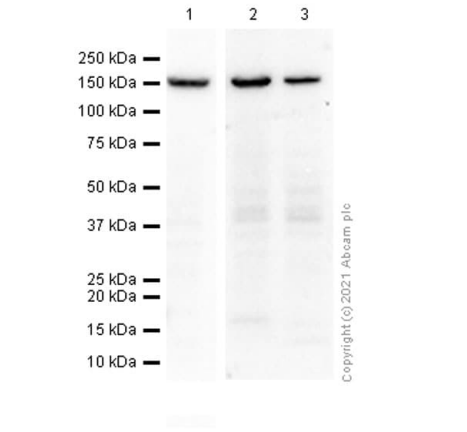
Lanes 1 - 4: Merged signal (red and green). Green - **ab134181** observed at 165 kDa. Red - loading control, **ab18058**, observed at 130 kDa.

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] - BSA and Azide free (ab230799)

This data was developed using **ab134181**, the same antibody clone in a different buffer formulation.
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**) 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] - BSA and Azide free (ab230799)

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

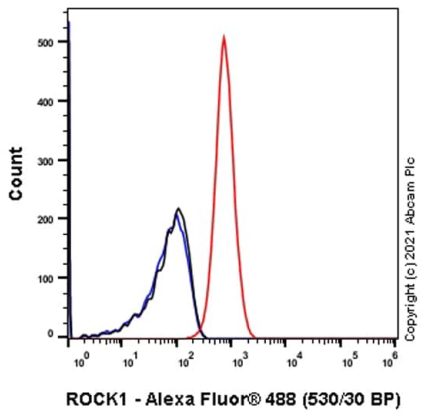
Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : Mouse brain
Lane 3 : Rat brain

Lysates/proteins at 20 µg per lane.

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

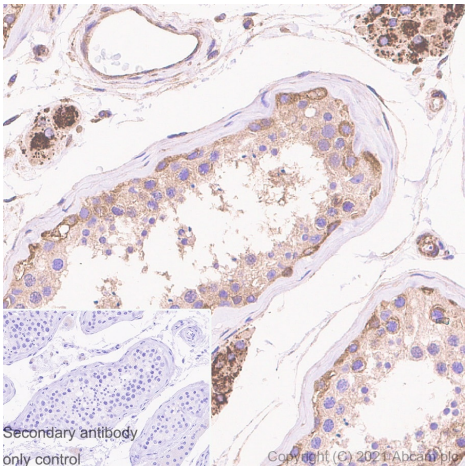
Predicted band size: 158 kDa
Observed band size: 158 kDa

This data was developed using **ab134181**, the same antibody clone in a different buffer formulation.



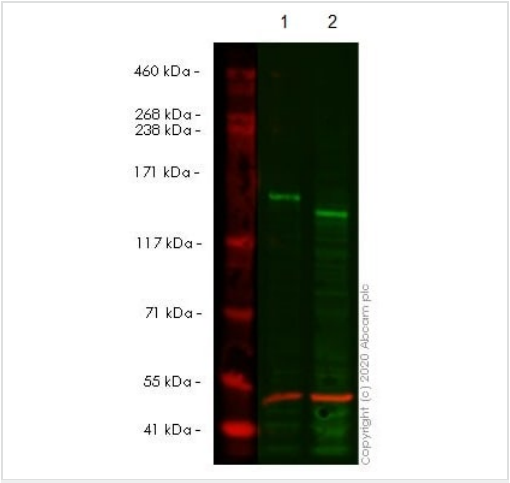
Flow Cytometry (Intracellular) - Anti-ROCK1 antibody [EPR638Y] - BSA and Azide free (ab230799)

This data was developed using **ab134181**, the same antibody clone in a different buffer formulation.
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] - BSA and Azide free (ab230799)

This data was developed using **ab134181**, the same antibody clone in a different buffer formulation.
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**) 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] - BSA and Azide free (ab230799)

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.

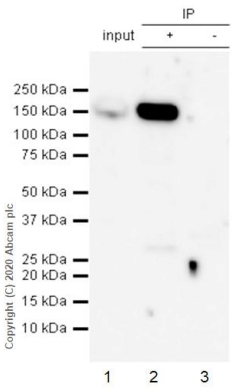
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (**ab134181**).

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] - BSA and Azide free (ab230799)

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
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab134181**).

Anti-ROCK1 antibody [EPR638Y] - BSA and Azide free (ab230799)

- 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
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