abcam

Product datasheet

Anti-ROCK2 + ROCK1 antibody [EP786Y] ab45171

Recombinant RabMAb

9 Abreviews 126 References 9 Images

Overview

Product name	Anti-ROCK2 + ROCK1 antibody [EP786Y]
Description	Rabbit monoclonal [EP786Y] to ROCK2 + ROCK1
Host species	Rabbit
Specificity	This antibody recognizes both the cleaved C-terminus of ROCK 1 (30 kDa) and full length protein (158 kDa). The immunogen used for this product shares 83% homology with ROCK2 and has been shown to bind recombinant human ROCK2, please see western blot images below.
Tested applications	**Suitable for:** Flow Cyt (Intra), IHC-Fr, WB, IHC-P, ICC/IF, IP
Species reactivity	**Reacts with:** Mouse, Rat, Cow, Human
Immunogen	Synthetic peptide within Human ROCK1 aa 1100-1200 (C terminal). The exact sequence is proprietary. Database link: **Q13464**
Positive control	WB: Untreated, Calyculin A treated and Camptothecin treated HeLa whole cell lysate (**ab150035**). Jurkat, Ramos, PC-12 and RAW264.7 cell lysates. IHC-P: Human adenocarcinoma of the colon and thyroid gland carcinoma tissues. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa cells. IP: HeLa whole cell lysate (**ab150035**).
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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20

Preservative: 0.01% Sodium azide
Constituents: PBS, 40% Glycerol, 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP786Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab45171 in the following tested applications.

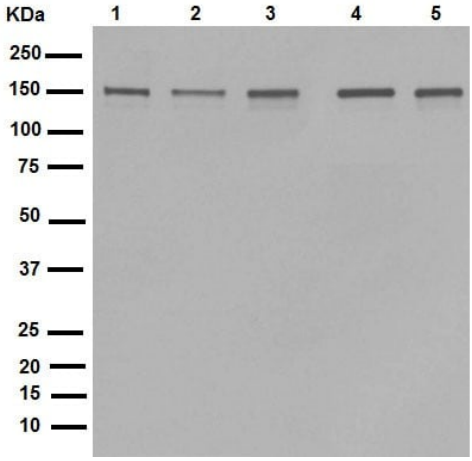
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/1000. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-Fr		Use at an assay dependent concentration. PubMed: 19295659
WB	★★★★☆ (4)	1/2000 - 1/10000. Predicted molecular weight: 158 kDa. **For unpurified use at 1/500.**
IHC-P	★★★★★ (2)	1/50 - 1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
ICC/IF	★☆☆☆☆ (1)	1/50 - 1/500.
IP		1/40 - 1/50.

Target

Cellular localization ROCK2: Cytoplasm. Cell membrane. Cytoplasmic, and associated with actin microfilaments and the plasma membrane. ROCK1: 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-ROCK2 + ROCK1 antibody [EP786Y] (ab45171)

All lanes : Anti-ROCK2 + ROCK1 antibody [EP786Y] (ab45171) at 1/5000 dilution (purified)

Lane 1 : HeLa cell lysate - treated with Calyculin A
Lane 2 : HeLa cell lysate - treated with Camptothecin
Lane 3 : HeLa cell lysate
Lane 4 : Jurkat cell lysate
Lane 5 : Ramos cell lysate

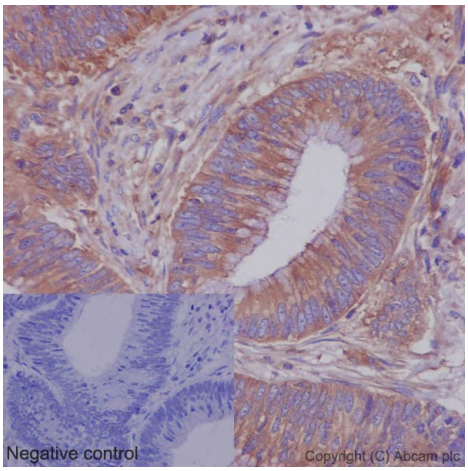
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

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

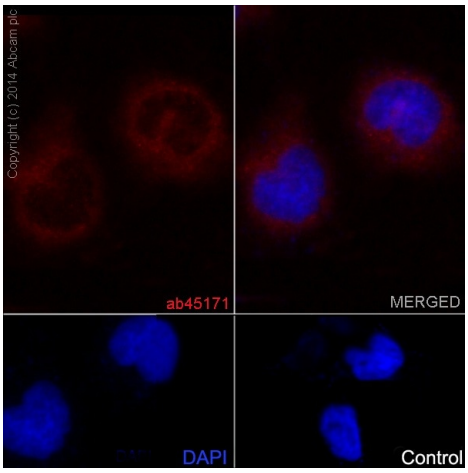
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ROCK2 + ROCK1 antibody [EP786Y] (ab45171)

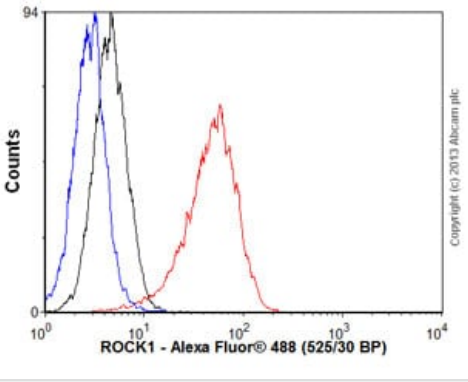
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human adenocarcinoma of the colon tissue labelling ROCK2 + ROCK1 with purified ab45171 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit IgG was used as the secondary antibody. Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunocytochemistry/ Immunofluorescence - Anti-ROCK2 + ROCK1 antibody [EP786Y] (ab45171)

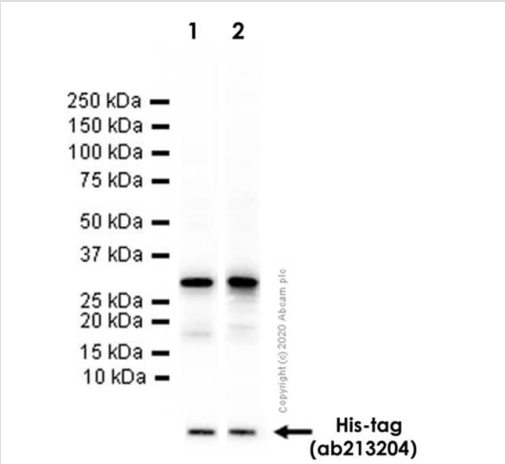
Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling ROCK2 + ROCK1 with purified ab45171 at 1/50. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150078**, an Alexa Fluor® 555-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain.

Control: primary antibody (1/50) and secondary antibody, **ab150113**, an Alexa Fluor® 488-conjugated goat anti-mouse IgG (1/500).



Flow Cytometry (Intracellular) - Anti-ROCK2 + ROCK1 antibody [EP786Y] (ab45171)

Overlay histogram showing HeLa cells stained with unpurified ab45171 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab45171, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluorr® 488 goat anti-rabbit IgG (H+L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Western blot - Anti-ROCK2 + ROCK1 antibody [EP786Y] (ab45171)

All lanes : Anti-ROCK2 + ROCK1 antibody [EP786Y] (ab45171) at 1/1000 dilution

Lane 1 : Recombinant Human ROCK1 protein (aa 1114 to 1354) (30 kDa)
Lane 2 : Recombinant Human ROCK2 protein (aa 1132 to 1388) (30 kDa)

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

Predicted band size: 158 kDa
Observed band size: 30 kDa

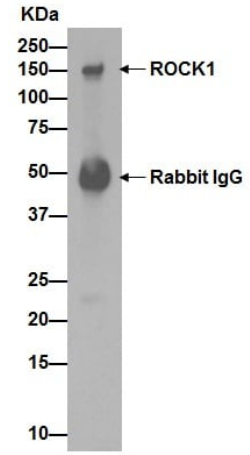
Loading control: Anti-6X His tag® antibody [EPR20547] (**ab213204**)

Blocking buffer and concentration: 5% NFDM/TBST

Exposure Times:

Lane 1: 3 seconds

Lane 2: 7 seconds

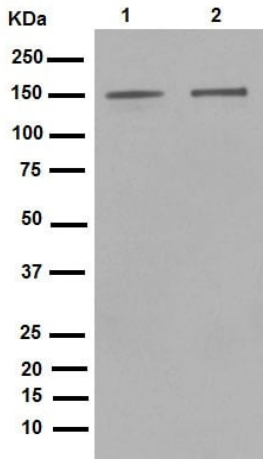


Immunoprecipitation - Anti-ROCK2 + ROCK1 antibody [EP786Y] (ab45171)

ab45171 (purified) at 1/40 immunoprecipitating ROCK2 + ROCK1 in HeLa cell lysate. For western blotting, a peroxidase-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/1000).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-ROCK2 + ROCK1 antibody [EP786Y] (ab45171)

All lanes : Anti-ROCK2 + ROCK1 antibody [EP786Y] (ab45171) at 1/5000 dilution (purified)

Lane 1 : PC-12 cell lysate
Lane 2 : RAW264.7 cell lysate

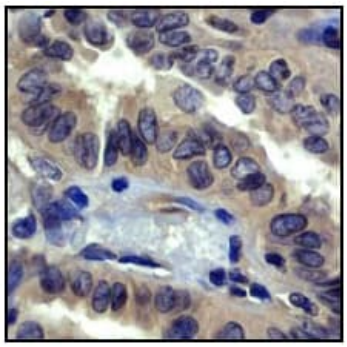
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

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

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ROCK2 + ROCK1 antibody [EP786Y] (ab45171)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human thyroid gland carcinoma tissue labelling ROCK2 + ROCK1 with unpurified ab45171.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Anti-ROCK2 + ROCK1 antibody [EP786Y] (ab45171)

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