# abcam

# Product datasheet

# Anti-ROCK2 + ROCK1 antibody [EP786Y] ab45171

重组 RabMAb

★★★★ 9 Abreviews 126 References 9图像

概述

产品名称 Anti-ROCK2 + ROCK1抗体[EP786Y]

描述 兔单克隆抗体[EP786Y] to ROCK2 + ROCK1

宿主 Rabbit

特异性 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.

经测试应用 适用于: Flow Cyt (Intra), IHC-Fr, WB, IHC-P, ICC/IF, IP

种属反应性 与反应: Mouse, Rat, Cow, Human

免疫原 Synthetic peptide within Human ROCK1 aa 1100-1200 (C terminal). The exact sequence is

proprietary.

Database link: Q13464

阳性对照 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).

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.

Avoid freeze / thaw cycle.

存储溶液 pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

纯**度** Protein A purified

**同种型** IgG

#### 应用

# The Abpromise guarantee Abpromise™承诺保证使用ab45171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。

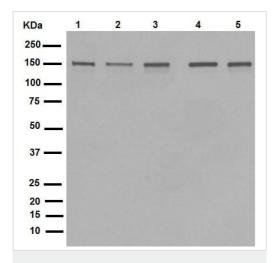
应用	Ab评论	说明
Flow Cyt (Intra)		1/1000. <b>ab172730</b> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
IHC-Fr		Use at an assay dependent concentration. PubMed: 19295659
WB	★★★★ 🚖 (4)	1/2000 - 1/10000. Predicted molecular weight: 158 kDa. For unpurified use at 1/500.
IHC-P	<b>★★★★★</b> (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	<b>★☆☆☆☆(1)</b>	1/50 - 1/500.
IP		1/40 - 1/50.

## 靶标

#### 细胞定位

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.

# 图片



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 lgG (H+L) at 1/1000 dilution

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

Blocking buffer and concentration: 5% NFDM/TBST.

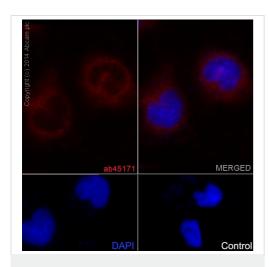
Diluting buffer and concentration: 5% NFDM /TBST.

Negative control

Gopyright (C) Abcam plg

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

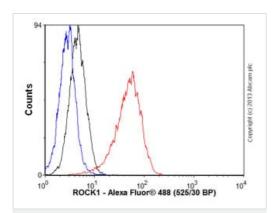
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human adenocarcinoma of the colon tissue labelling ROCK2 + ROCK1 with purified ab45171 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit lgG 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)

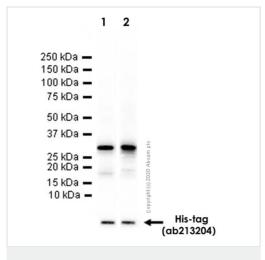
Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling ROCK2 + ROCK1 with purified ab45171 at 1/50. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150078**, an Alexa Fluor<sup>®</sup> 555-conjugated goat antirabbit lgG (1/500) was used as the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

Control: primary antibody (1/50) and secondary antibody, **ab150113**, an Alexa Fluor<sup>®</sup> 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 lgG (H+L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (0.1 $\mu$ g/1x10<sup>6</sup> 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 lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

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

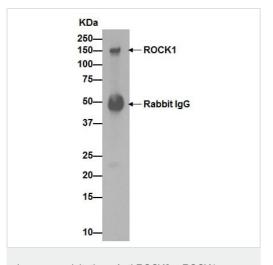
Loading control: Anti-6X His tag® antibody [EPR20547] (ab213204)

Blocking buffer and concentration: 5% NFDM/TBST

**Exposure Times:** 

Lane 1:3 seconds

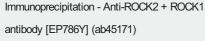
Lane 2: 7 seconds



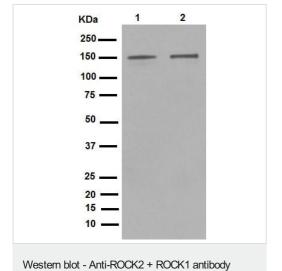
ab45171 (purified) at 1/40 immunoprecipitating ROCK2 + ROCK1 in HeLa cell lysate. For western blotting, a peroxidase-conjugated goat anti-rabbit lgG (H+L) was used as the secondary antibody (1/1000).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



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



[EP786Y] (ab45171)

Lane 1: PC-12 cell lysate

Lane 2: RAW264.7 cell lysate

Lysates/proteins at 20 µg per lane.

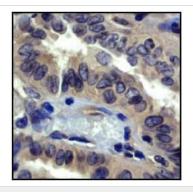
#### Secondary

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

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

Blocking buffer and concentration: 5% NFDM/TBST.

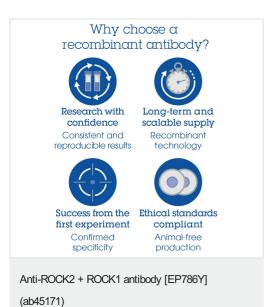
Diluting buffer and concentration: 5% NFDM /TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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.



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