# abcam

# Product datasheet

# Anti-ROCK2 antibody [EPR7141(B)] ab125025





★★★★ 4 Abreviews 39 References

8 Images

#### Overview

**Product name** Anti-ROCK2 antibody [EPR7141(B)]

**Description** Rabbit monoclonal [EPR7141(B)] to ROCK2

**Host species** Rabbit

**Tested applications** Suitable for: WB, ICC/IF

Unsuitable for: IHC-P

Species reactivity Reacts with: Mouse, Rat, Human

**Immunogen** Synthetic peptide within Human ROCK2 (C terminal). The exact sequence is proprietary.

Positive control HepG2, A549, HeLa, HEK293, L6, RAW264.7 and A673 cell lysates; Human kidney tissue,

Mouse and Rat brain tissue lysate.

**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**

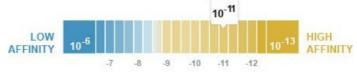
**Form** Liquid

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.

pH: 7.20

 $K_D = 8.70 \times 10^{-11} M$ Dissociation constant (K<sub>D</sub>)



Learn more about K<sub>D</sub>

Storage buffer

Preservative: 0.01% Sodium azide

Constituents: PBS, 0.14% BSA, 40% Glycerol (glycerin, glycerine)

Purity Protein A purified

ClonalityMonoclonalClone numberEPR7141(B)

**Isotype** IgG

#### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab125025 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	★★★★☆(3)	1/10000 - 1/50000. Detects a band of approximately 161 kDa (predicted molecular weight: 161 kDa).
ICC/IF		1/100 - 1/250.

**Application notes** Is unsuitable for IHC-P.

#### **Target**

**Function** Regulates the assembly of the actin cytoskeleton. Promotes formation of stress fibers and of focal

adhesion complexes. Plays a role in smooth muscle contraction.

**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.

Post-translational

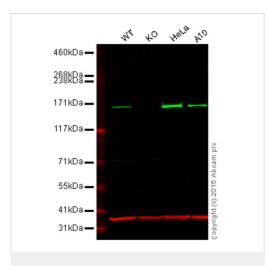
modifications

Phosphorylated upon DNA damage, probably by ATM or ATR.

Cytoplasm. Cell membrane. Cytoplasmic, and associated with actin microfilaments and the

plasma membrane.

### **Images**



Western blot - Anti-ROCK2 antibody [EPR7141(B)] (ab125025)

Lane 1: Wild-type HAP1 cell lysate (20 µg)

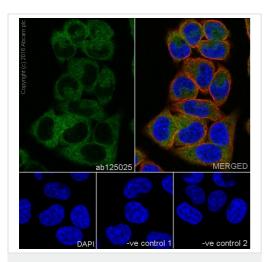
Lane 2: ROCK2 knockout HAP1 cell lysate (20 µg)

Lane 3: HeLa cell lysate (20 µg)

Lane 4: A10 cell lysate (20 µg)

**Lanes 1 - 4**: Merged signal (red and green). Green - ab125025 observed at 165 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

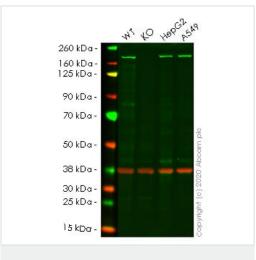
ab125025 was shown to specifically react with ROCK2 when ROCK2 knockout samples were used. Wild-type and ROCK2 knockout samples were subjected to SDS-PAGE. ab125025 and ab8245 (loading control to GAPDH) were diluted 1/10 000 and 1/2000 respectively and incubated overnight at 4°C. Blots were developed 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 1/10,000 dilution for 1 h at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-ROCK2 antibody [EPR7141(B)] (ab125025)

Immunocytochemistry/Immunofluorescence analysis of HeLa (human cervix adenocarcinoma) cells labelling ROCK2 with purified ab125025 at 1/250. Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. <a href="mailto:ab150077">ab150077</a>, Alexa Fluor<sup>®</sup> 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. Cells were counter-stained with <a href="mailto:ab7291">ab7291</a> anti-Tubulin (mouse mAb) primary and <a href="mailto:ab150120">ab150120</a> (AlexaFluor<sup>®</sup>594 goat anti-mouse) secondary both at 1/1000 dilution. Nuclei were counterstained with DAPI (blue).

For negative control 1, rabbit primary antibody and <u>ab150120</u> (antimouse) secondary antibody were used. For negative control 2, <u>ab7291</u> (mouse primary antibody) was used followed by <u>ab150077</u> (anti-rabbit secondary antibody).



Western blot - Anti-ROCK2 antibody [EPR7141(B)] (ab125025)

**All lanes :** Anti-ROCK2 antibody [EPR7141(B)] (ab125025) at 1/10000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: ROCK2 knockout HeLa cell lysate

Lane 3: HepG2 cell lysate

Lane 4: A549 cell lysate

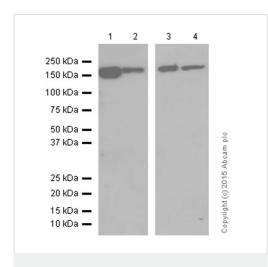
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 161 kDa **Observed band size:** 175 kDa

**Lanes 1-4:** Merged signal (red and green). Green - ab125025 observed at 175 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab125025 was shown to react with ROCK2 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab265679 (knockout cell lysate ab257643) was used. Wild-type HeLa and ROCK2 knockout 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. ab125025 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 10000 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 (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-ROCK2 antibody [EPR7141(B)] (ab125025)

**All lanes :** Anti-ROCK2 antibody [EPR7141(B)] (ab125025) at 1/50000 dilution

Lane 1: HeLa whole cell lysate

Lane 2: HEK293 whole cell lysate

Lane 3: Mouse brain tissue lysate

Lane 4: Rat brain tissue lysate

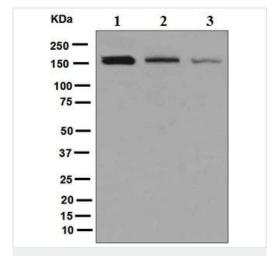
Lysates/proteins at 10 µg per lane.

## Secondary

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

dilution

**Predicted band size:** 161 kDa **Observed band size:** 161 kDa



Western blot - Anti-ROCK2 antibody [EPR7141(B)] (ab125025)

Blocking and Diluting buffer 5% NFDM/TBST

**All lanes :** Anti-ROCK2 antibody [EPR7141(B)] (ab125025) at 1/10000 dilution (unpurified)

Lane 1 : Hela cell lysate

Lane 2 : L6 cell lysate

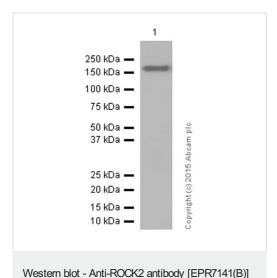
Lane 3: A673 cell lysate

Lysates/proteins at 10 µg per lane.

### Secondary

All lanes: HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 161 kDa



(ab125025)

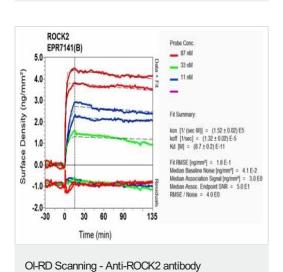
Anti-ROCK2 antibody [EPR7141(B)] (ab125025) at 1/10000 dilution + RAW264.7 whole cell lysate at 10  $\mu g$ 

# **Secondary**

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

**Predicted band size:** 161 kDa **Observed band size:** 161 kDa

Blocking and Diluting buffer 5% NFDM/TBST



[EPR7141(B)] (ab125025)

Equilibrium disassociation constant ( $K_D$ ) Learn more about  $K_D$ 

Click here to learn more about K<sub>D</sub>



Anti-ROCK2 antibody [EPR7141(B)] (ab125025)

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