

Product datasheet

Anti-ROCK2 antibody [EPR7141(B)] - BSA and Azide free ab238961

KO VALIDATED Recombinant RobMAb

5 Images

Overview		
Product name	Anti-ROCK2 antibody [EPR7141(B)] - BSA and Azide free	
Description	Rabbit monoclonal [EPR7141(B)] to ROCK2 - BSA and Azide free	
Host species	Rabbit	
Tested applications	Suitable for: WB, ICC/IF Unsuitable for: IHC-P	
Species reactivity	Reacts with: Mouse, Rat, Human	
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.	
Positive control	WB: HeLa, HepG2, A10 and HAP1 cell lysate. ICC: HeLa cells.	
General notes	ab238961 is the carrier-free version of <u>ab125025</u> .	
	Our <u>carrier-free</u> 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 <u>conjugation kits</u> 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.	
	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. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR7141(B)
lsotype	lgG

Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab238961 in the following tested applications.

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

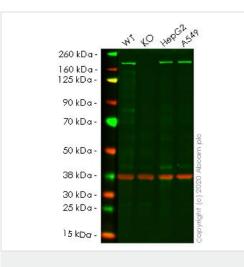
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 161 kDa (predicted molecular weight: 161 kDa).
ICC/IF		Use at an assay dependent concentration.

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



Western blot - Anti-ROCK2 antibody [EPR7141(B)] -BSA and Azide free (ab238961)

All lanes : Anti-ROCK2 antibody [EPR7141(B)] (<u>ab125025</u>) 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.

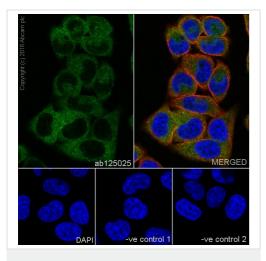
Performed under reducing conditions.

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

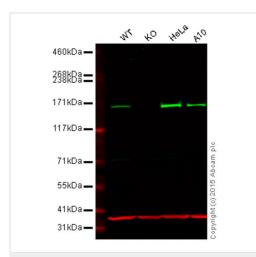
This data was developed using the same antibody clone in a different buffer formulation (<u>ab125025</u>).

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



Immunocytochemistry/ Immunofluorescence - Anti-ROCK2 antibody [EPR7141(B)] - BSA and Azide free (ab238961)



Western blot - Anti-ROCK2 antibody [EPR7141(B)] -BSA and Azide free (ab238961)

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. **ab150077**, Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. Cells were counter-stained with **ab7291** anti-Tubulin (mouse mAb) primary and **ab150120** (AlexaFluor[®]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).

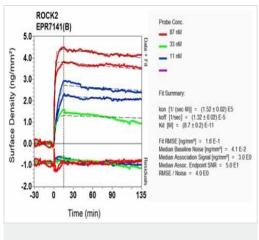
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab125025</u>).

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 - <u>ab125025</u> 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. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol and

sodium azide (ab125025).



OI-RD Scanning - Anti-ROCK2 antibody

[EPR7141(B)] - BSA and Azide free (ab238961)



Azide free (ab238961)

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Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about KD

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab125025**).

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