abcam

Product datasheet

Anti-ROCK2 antibody ab71598

KO VALIDATED

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Overview

Product name	Anti-ROCK2 antibody
Description	Rabbit polyclonal to ROCK2
Host species	Rabbit
Tested applications	**Suitable for:** IP, ICC/IF, IHC-Fr, WB, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Cow, Chimpanzee, Rhesus monkey
Immunogen	Synthetic peptide conjugated to KLH derived from within residues 1 - 100 of Human ROCK2.Read Abcam's proprietary immunogen policy(Peptide available as ab71597.)
Positive control	WB: Mouse and rat liver tissue lysates and SK N SH, U2OS, HeLa whole cell lysates. IHC-P: Human liver cancer tissue. ICC/IF: Wild-type HAP1 cells.

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.02% Sodium Azide Constituents: 1% BSA, PBS, pH 7.4
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab71598** 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
IP		Use a concentration of 5 µg/ml.
ICC/IF		1/250.
IHC-Fr	★★★★☆	Use at an assay dependent concentration.
WB	★★★★★	Use a concentration of 1 µg/ml. Detects a band of approximately 171 kDa (predicted molecular weight: 161 kDa).Can be blocked with Human ROCK2 peptide (ab71597).
IHC-P		Use a concentration of 5 µg/ml.

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.

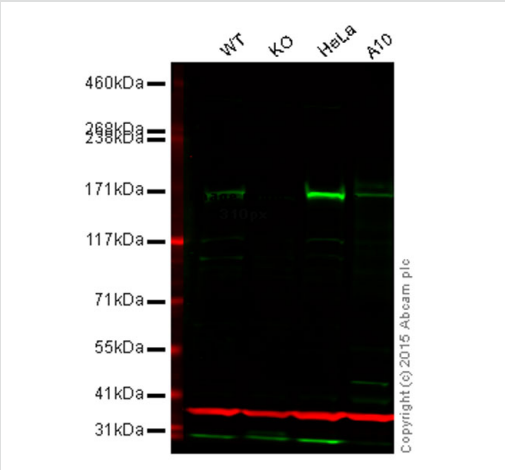
Post-translational modifications

Phosphorylated upon DNA damage, probably by ATM or ATR.

Cellular localization

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

Images

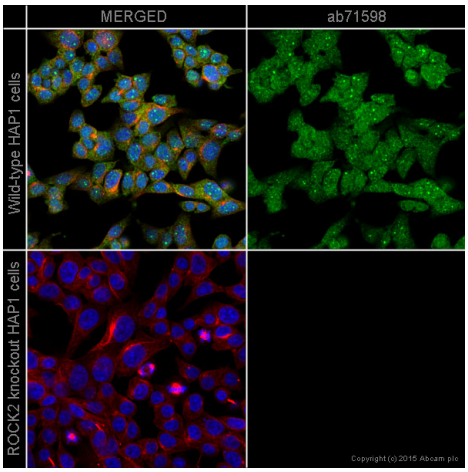


Western blot - Anti-ROCK2 antibody (ab71598)

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 - ab71598 observed at 171 kDa. Red - loading control, ab8245, observed at 37 kDa.

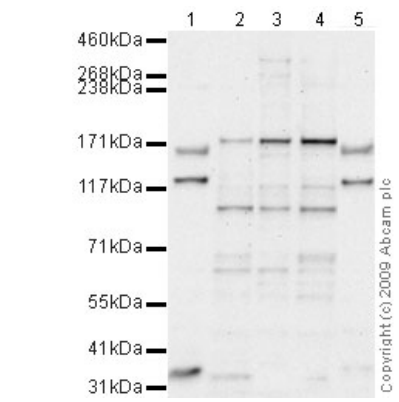
ab71598 was shown to recognize ROCK2 when ROCK2 knockout samples were used, along with additional cross-reactive bands. Wild-type and ROCK2 knockout samples were subjected to SDS-PAGE. ab71598 and ab8245 (loading control to GAPDH) were diluted 1 µg/mL and 1/2000 respectively and incubated overnight at 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 1/10000 dilution for 1 h at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-ROCK2 antibody (ab71598)

ab71598 staining ROCK2 in wild-type HAP1 cells (top panel) and ROCK2 knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab71598 at 1/250 dilution and ab195889 at 1/250 dilution (shown in pseudo colour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.



Western blot - Anti-ROCK2 antibody (ab71598)

All lanes : Anti-ROCK2 antibody (ab71598) at 1 µg/ml

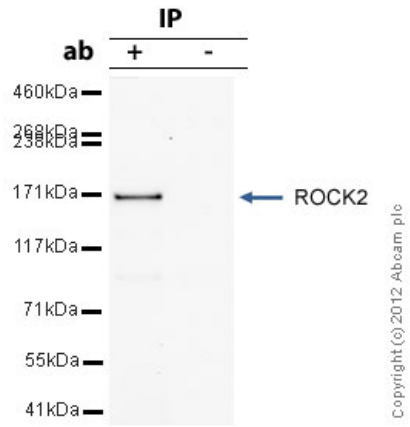
Lane 1 : Liver (Mouse) Tissue Lysate
Lane 2 : SK N SH (Human neuroblastoma) Whole Cell Lysate
Lane 3 : U2OS (Human osteosarcoma cell line) Whole Cell Lysate
Lane 4 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 5 : Liver (Rat) Tissue Lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

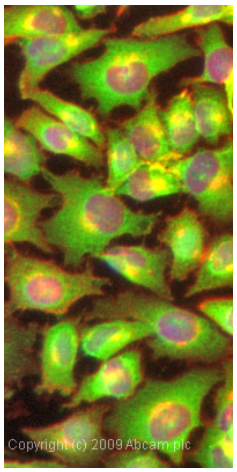
Performed under reducing conditions.

Predicted band size: 161 kDa
Observed band size: 171 kDa
why is the actual band size different from the predicted?
Additional bands at: 100 kDa, 125 kDa. We are unsure as to the identity of these extra bands.



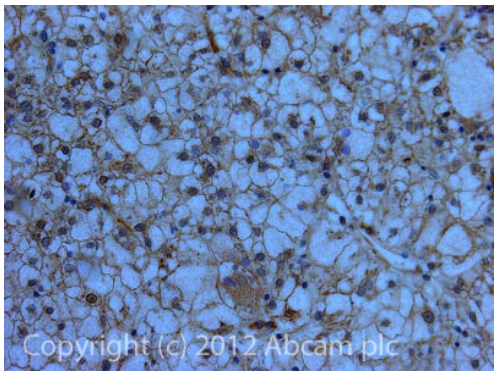
Immunoprecipitation - Anti-ROCK2 antibody (ab71598)

ROCK2 was immunoprecipitated using 0.5mg Hela whole cell extract, 5µg of Rabbit polyclonal to ROCK2 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-). The antibody was incubated under agitation with Protein G beads for 10min, Hela whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70^{o}C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab71598.
Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (ab99697).
Band: 171kDa; ROCK2



Immunocytochemistry/ Immunofluorescence - Anti-ROCK2 antibody (ab71598)

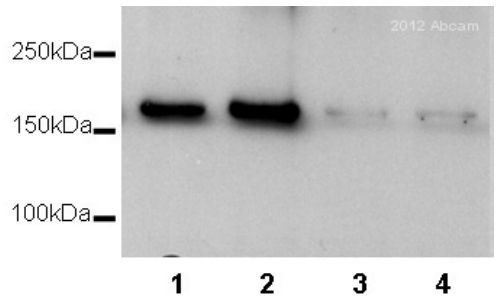
ICC/IF image of ab71598 stained HeLa cells. The cells were 10% neutral buffered formalin fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab71598, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ROCK2 antibody (ab71598)

IHC image of ROCK2 staining in Human liver cancer formalin fixed paraffin embedded tissue section, performed on a Leica BondTM system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab71598, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Western blot - Anti-ROCK2 antibody (ab71598)
This image is courtesy of an anonymous Abreview

All lanes : Anti-ROCK2 antibody (ab71598) at 1/1000 dilution

Lanes 1-2 : Human primary myometrial whole cell lysate with scrambled siRNA
Lanes 3-4 : Human primary myometrial whole cell lysate with siRNA against ROCK2

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : HRP conjugated Goat anti-rabbit IgG polyclonal at 1/4000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 161 kDa
Observed band size: 160-170 kDa why is the actual band size different from the predicted?

Exposure time: 2 minutes

Blocked with 5% BSA for 1 hour at 20°C

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