abcam

ab309104

Anti-RBCK1 antibody [EPR28157-53]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR28157-53
Purification technique	Affinity purification Protein A
Concentration	0.507 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Species	Mouse

Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/50
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Mouse
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/500
Notes	-

Species	Mouse
Dilution info	1/500

Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Not recommended

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

IP

Not recommended

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

Target data

See full target information RBCK1

Function

E3 ubiquitin-protein ligase, which accepts ubiquitin from specific E2 ubiquitin-conjugating enzymes, such as UBE2L3/UBCM4, and then transfers it to substrates (PubMed:12629548, PubMed:17449468, PubMed:18711448). Functions as an E3 ligase for oxidized IREB2 and both heme and oxygen are necessary for IREB2 ubiquitination (PubMed:12629548). Promotes ubiquitination of TAB2 and IRF3 and their degradation by the proteasome (PubMed:17449468, PubMed:18711448). Component of the LUBAC complex which conjugates linear ('Met-1'-linked) polyubiquitin chains to substrates and plays a key role in NF-kappa-B activation and regulation of inflammation (PubMed:17006537, PubMed:19136968, PubMed:21455173, PubMed:21455180, PubMed:21455181). LUBAC conjugates linear polyubiquitin to IKBKG and RIPK1 and is involved in activation of the canonical NF-kappa-B and the JNK signaling pathways (PubMed:17006537, PubMed:19136968, PubMed:21455173, PubMed:21455180, PubMed:21455181). Linear ubiquitination mediated by the LUBAC complex interferes with TNF-induced

cell death and thereby prevents inflammation (PubMed:17006537, PubMed:21455173, PubMed:21455180, PubMed:21455181). LUBAC is recruited to the TNF-R1 signaling complex (TNF-RSC) following polyubiquitination of TNF-RSC components by BIRC2 and/or BIRC3 and to conjugate linear polyubiquitin to IKBKG and possibly other components contributing to the stability of the complex (PubMed:17006537, PubMed:19136968, PubMed:21455173, PubMed:21455180, PubMed:21455181). The LUBAC complex is also involved in innate immunity by conjugating linear polyubiquitin chains at the surface of bacteria invading the cytosol to form the ubiquitin coat surrounding bacteria (PubMed:28481331). LUBAC is not able to initiate formation of the bacterial ubiquitin coat, and can only promote formation of linear polyubiquitins on pre-existing ubiquitin (PubMed:28481331). The bacterial ubiquitin coat acts as an 'eat-me' signal for xenophagy and promotes NF-kappa-B activation (PubMed:28481331). Together with OTULIN, the LUBAC complex regulates the canonical Wnt signaling during angiogenesis (PubMed:23708998). Binds polyubiquitin of different linkage types (PubMed:20005846, PubMed:21455181).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

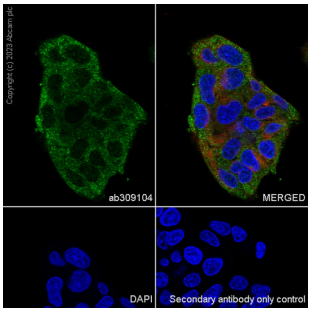
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

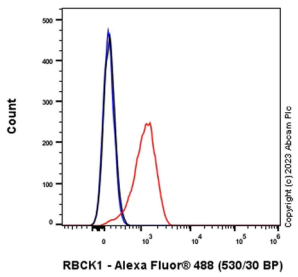
Full details and terms and conditions can be found here:
Terms & Conditions.

7 product images



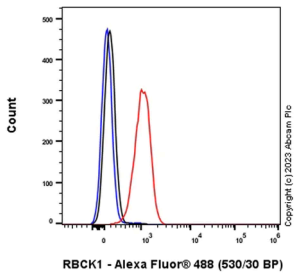
Immunocytochemistry/ Immunofluorescence - Anti-RBCK1 antibody [EPR28157-53] (ab309104)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HepG2 (human hepatocellular carcinoma epithelial cell) cells labelling RBCK1 with ab309104 at 1/50 (10.14 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 2 ug/ml dilution (Green). Confocal image showing mostly cytoplasmic staining in HepG2 cell line. Image was taken with a confocal microscope(Leica-Microsystems, TCS SP8). ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/ml dilution (Red). The Nuclear counterstain was DAPI (Blue). Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/ml dilution.



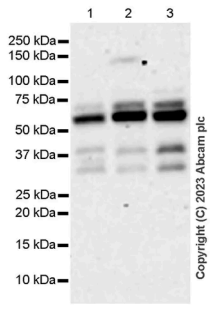
Flow Cytometry (Intracellular) - Anti-RBCK1 antibody [EPR28157-53] (ab309104)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HepG2 (human hepatocellular carcinoma epithelial cell) cells labelling RBCK1 with ab309104 at 1/500 dilution (0.1 ug)/Red (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-RBCK1 antibody [EPR28157-53] (ab309104)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized NIH/3T3 (mouse embryonic fibroblast) cells labelling RBCK1 with ab309104 at 1/500 dilution (0.1 ug)/Red (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



Western blot - Anti-RBCK1 antibody [EPR28157-53] (ab309104)

Blocking and diluting buffer and concentration: 5% NFDM/TBST. The expression molecular weight observed is consistent with what has been described in the literature (PMID: 31209050, PMID: 27006117). The higher MW(57kd) band is the ubiquitylated form of RBCK1(PMID: 31209050). Bands below 57kd are cleaved RBCK1(PMID: 27006117).
All lanes:
Western blot - Anti-RBCK1 antibody [EPR28157-53] (ab309104) at 1/1000 dilution

Lane 1:
A549 (human lu carcinoma epithelial cell)whole cell lysate at 20 µg

Lane 2:
NIH/3T3 (mouse embryonic fibroblast) whole cell lysate at 20 µg

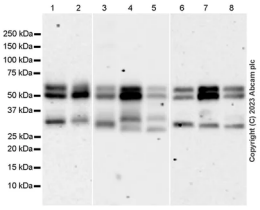
Lane 3:
C2C12 (mouse myoblast) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 57 kDa

Exposure time: 180s



Western blot - Anti-RBCK1 antibody [EPR28157-53] (ab309104)

Blocking and diluting buffer and concentration: 5% NFDM/TBST The expression molecular weight observed is consistent with what has been described in the literature (PMID: 31209050, PMID: 27006117). The higher MW(57kd) band is the ubiquitylated form of RBCK1(PMID: 31209050). Bands below 57kd are cleaved RBCK1(PMID: 27006117). The blot of lanes1, 2 and 6-8 were developed

using a high sensitivity ECL substrate. Exposure time: Lanes1, 2 and 6-8: 180 seconds; Lane3-5: 48 seconds
All lanes:
Western blot - Anti-RBCK1 antibody [EPR28157-53] (ab309104) at 1/1000 dilution

Lane 1:
Human hypothalamus tissue lysate at 20 µg

Lane 2:
Human spinal cord tissue lysate at 20 µg

Lane 3:
Mouse brain tissue lysate at 20 µg

Lane 4:
Mouse spleen tissue lysate at 20 µg

Lane 5:
Mouse kidney tissue lysate at 20 µg

Lane 6:
Rat brain tissue lysate at 20 µg

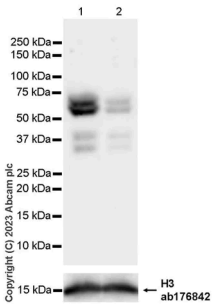
Lane 7:
Rat spleen tissue lysate at 20 µg

Lane 8:
Rat kidney tissue lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 57 kDa



Western blot - Anti-RBCK1 antibody [EPR28157-53] (ab309104)

Blocking and diluting buffer and concentration: 5% NFDM/TBST. The expression molecular weight observed is consistent with what has been described in the literature (PMID: 31209050, PMID: 27006117). The higher MW(57kd) band is the ubiquitylated form of RBCK1(PMID: 31209050). Bands below 57kd are cleaved RBCK1(PMID: 27006117). In Western blot, anti- H3 antibody (ab176842) loading control staining at 1/100000 dilution.
All lanes:
Western blot - Anti-RBCK1 antibody [EPR28157-53] (ab309104) at 1/1000 dilution

Lane 1:
HepG2 (human hepatocellar carcinoma epithelial cell) whole cell lysate at 20 µg

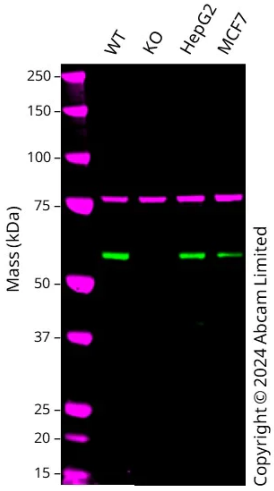
Lane 2:
HepG2 transfected with siRNA specifically targeti RBCK1 whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 57 kDa

Exposure time: 59s



Western blot - Anti-RBCK1 antibody [EPR28157-53] (ab309104)

RBCK1 Western blot staining using rabbit Anti-RBCK1 antibody

Western blot: Anti-RBCK1 antibody [EPR28157-53] (ab309104) staining at 1/1000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (ab238078) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab309104 was shown to bind specifically to RBCK1. A band was observed at 58 kDa in wild-type A549 cell lysates with no signal observed at this size in RBCK1 knockout cell line. To generate this image, wild-type and RBCK1 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-RBCK1 antibody [EPR28157-53] (ab309104) at 1/1000 dilution

Lane 1:
Wild-type A549 cell lysate at 20 µg

Lane 2:
RBCK1 knockout A549 cell lysate at 20 µg

Lane 3:
HepG2 cell lysate at 20 µg

Lane 4:
MCF7 cell lysate at 20 µg

Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Observed band size: 58 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.