abcam

ab326338

Anti-Kelch-like ECH-associated protein 1 antibody [EPR31136-529]

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	EPR31136-529
Purification technique	Affinity purification Protein A
Concentration	0.515 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/500
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/50
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/100
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Target data

See full target information KEAP1

Function

Substrate-specific adapter of a BCR (BTB-CUL3-RBX1) E3 ubiquitin ligase complex that regulates the response to oxidative stress by targeting NFE2L2/NRF2 for ubiquitination (PubMed:14585973, PubMed:15379550, PubMed:15572695, PubMed:15601839, PubMed:15983046, PubMed:37339955). KEAP1 acts as a key sensor of oxidative and electrophilic stress: in normal conditions, the BCR(KEAP1) complex mediates ubiquitination and degradation of NFE2L2/NRF2, a transcription factor regulating expression of many cytoprotective genes (PubMed:15601839, PubMed:16006525). In response to oxidative stress, different electrophile metabolites trigger non-enzymatic covalent modifications of highly reactive cysteine residues in KEAP1, leading to inactivate the ubiquitin ligase activity of the BCR(KEAP1) complex, promoting NFE2L2/NRF2 nuclear accumulation and expression of phase II detoxifying enzymes (PubMed:16006525, PubMed:17127771, PubMed:18251510, PubMed:19489739, PubMed:29590092). In

response to selective autophagy, KEAP1 is sequestered in inclusion bodies following its interaction with SQSTM1/p62, leading to inactivation of the BCR(KEAP1) complex and activation of NFE2L2/NRF2 (PubMed:20452972). The BCR(KEAP1) complex also mediates ubiquitination of SQSTM1/p62, increasing SQSTM1/p62 sequestering activity and degradation (PubMed:28380357). The BCR(KEAP1) complex also targets BPTF and PGAM5 for ubiquitination and degradation by the proteasome (PubMed:15379550, PubMed:17046835).

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.

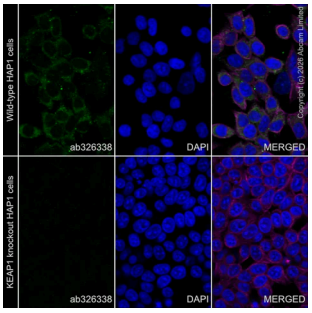
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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10 product images



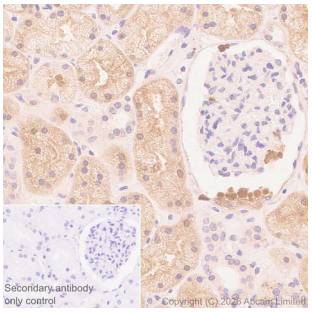
Immunocytochemistry/ Immunofluorescence - Anti-Kelch-like ECH-associated protein 1 antibody [EPR31136-529] (ab326338)

Kelch-like ECH-associated protein 1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Kelch-like ECH-associated protein 1 antibody

Immunofluorescent analysis of 100% methanol-fixed, 0.1% TritonX-100 permeabilized Parental HAP1 (human chronic myelogenous leukemia near-haploid cell) and KEAP1 KO HAP1 (KEAP1 knockout human chronic myelogenous leukemia near-haploid cell) cells labelling with ab326338 at 1/50 (9.82 µg/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (Green).

Confocal image showing cytoplasmic staining in parental HAP1 cell line and no staining in KEAP1 KO HAP1 cell line (shown in green). The counterstain was observed in magenta. Nuclear DNA was labelled with DAPI (shown in blue). Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

ab7291 Anti-alpha Tubulin mouse monoclonal antibody was used to counterstain tubulin at 1/1000 dilution, followed by ab150120 Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) at 1/1000 dilution (Magenta). The Nuclear counterstain was DAPI (Blue).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Kelch-like ECH-associated protein 1 antibody [EPR31136-529] (ab326338)

Kelch-like ECH-associated protein 1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human kidney using rabbit Anti-Kelch-like ECH-associated protein 1 antibody

Immunohistochemical analysis of paraffin-embedded Human kidney tissue labeling with ab326338 at 1/100 (4.91 µg/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on human kidney.

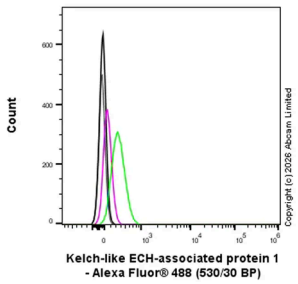
The primary antibody was incubated for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins

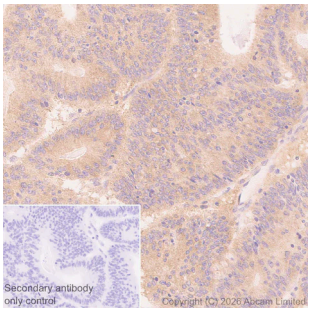


Flow Cytometry (Intracellular) - Anti-Kelch-like ECH-associated protein 1 antibody [EPR31136-529] (ab326338)

Kelch-like ECH-associated protein 1 Flow Cytometry (Intracellular) staining using rabbit Anti-Kelch-like ECH-associated protein 1 antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Parental HAP1(HOWT01) (human chronic myelogenous leukemia near-haploid cell) (Green and black) and KEAP1 KO HAP1(HO010289) (Magenta and grey) cells labelling with ab326338 at 1/500 dilution (0.1ug), compared with a Rabbit monoclonal IgG (ab172730) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody).

Goat anti-Rabbit IgG (Alexa Fluor® 647, ab150083) at 1/5000 dilution was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Kelch-like ECH-associated protein 1 antibody [EPR31136-529] (ab326338)

Kelch-like ECH-associated protein 1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human colon carcinoma using rabbit Anti-Kelch-like ECH-associated protein 1 antibody

Immunohistochemical analysis of paraffin-embedded Human colon carcinoma tissue labeling with ab326338 at 1/100 (4.91 µg/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on human colon carcinoma.

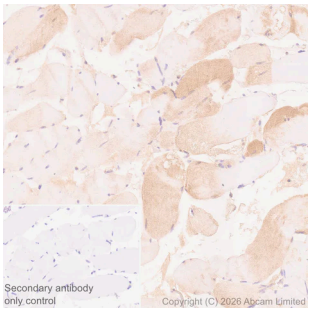
The primary antibody was incubated for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Kelch-like ECH-associated protein 1 antibody [EPR31136-529] (ab326338)

Kelch-like ECH-associated protein 1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human skeletal muscle using rabbit Anti-Kelch-like ECH-associated protein 1 antibody

Immunohistochemical analysis of paraffin-embedded Human skeletal muscle tissue labeling with ab326338 at 1/100 (4.91 µg/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on human skeletal msucle.

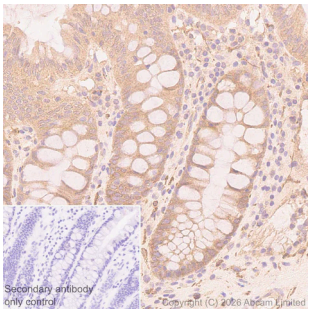
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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Kelch-like ECH-associated protein 1 antibody [EPR31136-529] (ab326338)

Kelch-like ECH-associated protein 1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human colon using rabbit Anti-Kelch-like ECH-associated protein 1 antibody

Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling with ab326338 at 1/100 (4.91 µg/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on human colon.

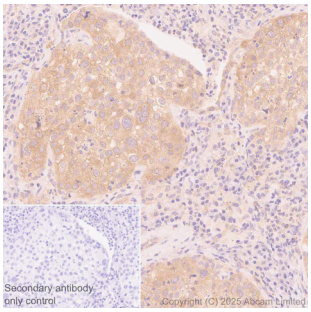
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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Kelch-like ECH-associated protein 1 antibody [EPR31136-529] (ab326338)

Kelch-like ECH-associated protein 1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human lung carcinoma using rabbit Anti-Kelch-like ECH-associated protein 1 antibody

Immunohistochemical analysis of paraffin-embedded Human lung carcinoma tissue labeling with ab326338 at 1/100 (4.91 µg/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on human lung carcinoma.

The primary antibody was incubated for 30 mins at room temperature.

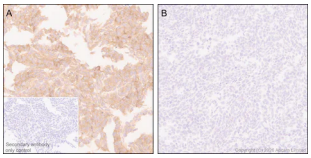
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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Kelch-like ECH-associated protein 1 antibody [EPR31136-529] (ab326338)

Kelch-like ECH-associated protein 1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Kelch-like ECH-associated protein 1 antibody

Immunohistochemical analysis of paraffin-embedded (A) Wild-type HAP1 (human chronic myelogenous leukemia near-haploid cell) cell pellet and (B) KEAP1 knockout HAP1 cell pellet tissue labeling with ab326338 at 1/200 (2.455 µg/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on (A) Wild-type HAP1 cell pellet, no staining on (B) KEAP1 knockout HAP1 cell pellet.

The primary antibody was incubated for 30 mins at room temperature.

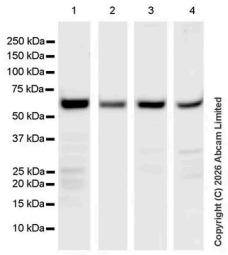
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins

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Western blot - Anti-Kelch-like ECH-associated protein 1 antibody [EPR31136-529] (ab326338)

Kelch-like ECH-associated protein 1 Western blot staining using rabbit Anti-Kelch-like ECH-associated protein 1 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

All lanes:
Western blot - Anti-Kelch-like ECH-associated protein 1 antibody [EPR31136-529] (ab326338) at 1/1000 dilution

Lane 1:
Human lung cancer tissue lysate at 60 µg

Lane 2:
Human pancreas tissue lysate at 60 µg

Lane 3:
Human colon cancer tissue lysate at 60 µg

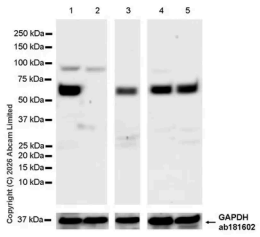
Lane 4:
Human breast cancer tissue lysate at 60 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Observed band size: 70 kDa

Exposure time: 37s



Western blot - Anti-Kelch-like ECH-associated protein 1 antibody [EPR31136-529] (ab326338)

Kelch-like ECH-associated protein 1 Western blot staining using rabbit Anti-Kelch-like ECH-associated protein 1 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

In Western blot, ab326338 was shown to bind specifically to KEAP1.
Target of interest was observed at 70 kDa in wild-type HAP1 cell lysates (lane 1) with no signal observed at this size in HAP1 knockout cell line (lane 2).

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) (1:200000) (36KDa).

All lanes:
Western blot - Anti-Kelch-like ECH-associated protein 1 antibody [EPR31136-529] (ab326338) at 1/1000 dilution

Lane 1:
Wild-type HAP-1 (human chronic myelogenous leukemia near-haploid cell) whole cell lysate at 60 µg

Lane 2:
KEAP1 knockout HAP1 whole cell lysate at 60 µg

Lane 3:

MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysate at 60 µg

Lane 4:
Calu-1 (human lung epidermoid carcinoma epithelial cell) whole cell lysate at 60 µg

Lane 5:
NCI-H1299 (human lung carcinoma epithelial cell) whole cell lysate at 60 µg

Secondary

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

Observed band size: 70 kDa, 36 kDa

Exposure time: 37s

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