abcam

Product datasheet

Anti-Beclin 1 antibody [EPR20473] ab210498

Recombinant RabMAb

105 References 10 Images

Overview

Product name Anti-Beclin 1 antibody [EPR20473]

Description Rabbit monoclonal [EPR20473] to Beclin 1

Host species Rabbit

Specificity We don't recommend this antibody for mouse and rat in IHC. In our hands most mouse and rat

tissues showed nuclear staining.

Tested applications Suitable for: IP, IHC-P, WB

Unsuitable for: Flow Cyt

Species reactivity Reacts with: Mouse, Rat, Human

Recombinant fragment. This information is proprietary to Abcam and/or its suppliers. **Immunogen**

Positive control WB: HeLa, HepG2, HEK-293, T-47D, Raji, RAW 264.7, NIH/3T3, A20 and C2C12 whole cell

> lysates; Human fetal brain and colon lysates; Mouse brain and spleen lysates; Rat brain, kidney and spleen lysates. IHC-P: Human kidney and breast cancer tissues. IP: HepG2 whole cell 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 long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

ClonalityMonoclonalClone numberEPR20473

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab210498 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		1/30.
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Detects a band of approximately 52 kDa (predicted molecular weight: 52 kDa).

Application notes Is unsuitable for Flow Cyt.

Target

Function Plays a central role in autophagy (By similarity). May play a role in antiviral host defense. Protects

against infection by a neurovirulent strain of Sindbis virus.

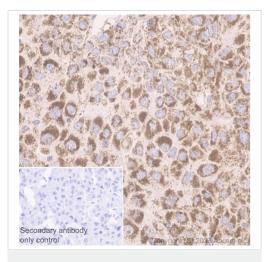
Tissue specificity Ubiquitous.

Sequence similarities Belongs to the beclin family.

Cellular localization Golgi apparatus > trans-Golgi network membrane. Interaction with ATG14 promotes translocation

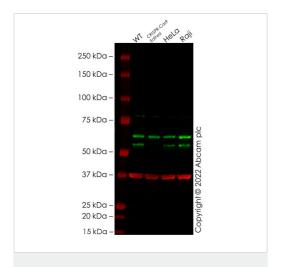
to autophagosomes. Expressed in dendrites and cell bodies of cerebellar Purkinje cells.

Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Beclin 1 antibody
[EPR20473] (ab210498)

Immunohistochemistry analysis of paraffin embedded sections of human hepatocellular carcinoma tissue staining Beclin 1 with ab210498 at 1/100 dilution and ready to use secondary Goat Anti-Rabbit IgG H&L (HRP polymer) (ab214880). Counterstained with hematoxylin. Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0). Positive staining on human hepatocellular carcinoma. The section was incubated with ab210498 at 4°C overnight.



Western blot - Anti-Beclin 1 antibody [EPR20473] (ab210498)

All lanes : Anti-Beclin 1 antibody [EPR20473] (ab210498) at 1/1000 dilution

Lane 1: Wild-type Jurkat cell lysate

Lane 2: BECN1 CRISPR-Cas9 edited Jurkat cell lysate

Lane 3 : HeLa cell lysate

Lane 4 : Raji cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 52 kDa
Observed band size: 52 kDa

False colour image of Western blot: Anti-Beclin 1 antibody
[EPR20473] staining at 1/1000 dilution, shown in green; Mouse
anti-GAPDH antibody [6C5] (ab8245) loading control staining at
1/20000 dilution, shown in red. In Western blot, ab210498 was
shown to bind specifically to Beclin 1. A band was observed at 52
kDa in wild-type Jurkat cell lysates with no signal observed at this
size in BECN1 CRISPR-Cas9 edited cell line ab283778 (BECN1
CRISPR-Cas9 edited cell lysate ab283792). The band observed in
the CRISPR-Cas9 edited lysate lane above 52 kDa is likely to
represent Beclin 1 with an insertion. This has not been investigated
further and the functional properties of the gene product have not
been determined. To generate this image, wild-type and BECN1

CRISPR-Cas9 edited Jurkat 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 lgG H&L 800CW and Goat anti-Mouse lgG H&L 680RD at 1/20000 dilution.

1 2 3 4 5

250 kDa —

150 kDa —

100 kDa —

75 kDa —

37 kDa —

25 kDa —

20 kDa —

15 kDa —

15 kDa —

10 kDa —

Western blot - Anti-Beclin 1 antibody [EPR20473] (ab210498)

All lanes : Anti-Beclin 1 antibody [EPR20473] (ab210498) at 1/1000 dilution

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2: HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate

Lane 3: HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

Lane 4 : T-47D (Human ductal breast epithelial tumor cell line) whole cell lysate

Lane 5: Raji (Human Burkitt's lymphoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

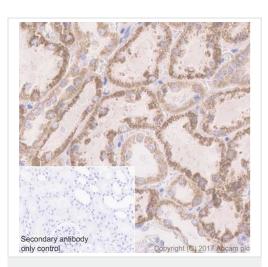
Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 52 kDa **Observed band size:** 52 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Beclin 1 antibody
[EPR20473] (ab210498)

Immunohistochemical analysis of paraffin-embedded human kidney tissue labeling Beclin 1 with ab210498 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

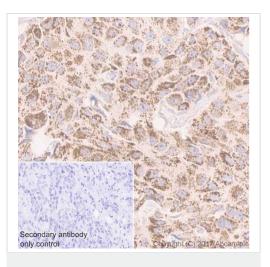
Granularly cytoplasmic staining on human kidney is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

We don't recommend this antibody for mouse and rat in IHC. In our hands most mouse and rat tissues showed nuclear staining.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Beclin 1 antibody
[EPR20473] (ab210498)

Immunohistochemical analysis of paraffin-embedded human breast cancer tissue labeling Beclin 1 with ab210498 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

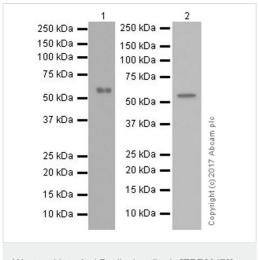
Granularly cytoplasmic staining on tumor cells of human breast cancer is observed.

Counter stained with Hematoxylin.

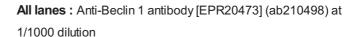
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

We don't recommend this antibody for mouse and rat in IHC. In our hands most mouse and rat tissues showed nuclear staining.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-Beclin 1 antibody [EPR20473] (ab210498)



Lane 1: Human fetal brain lysate

Lane 2: Human colon lysate

Lysates/proteins at 10 µg per lane.

Secondary

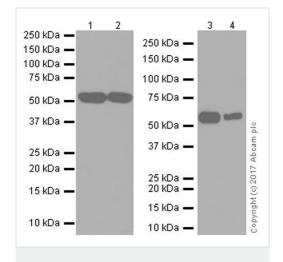
All lanes : VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) at 1/4000 dilution

Predicted band size: 52 kDa
Observed band size: 52 kDa

Observed band size: 52 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Beclin 1 antibody [EPR20473] (ab210498)

All lanes : Anti-Beclin 1 antibody [EPR20473] (ab210498) at 1/1000 dilution

Lane 1 : RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate at 10 μ g

Lane 2 : NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate at 10 µg

Lane 3 : A20 (Mouse reticulum sarcoma cell line) whole cell lysate at 10 μg

Lane 4: C2C12 (Mouse myoblast cell line) whole cell lysate

Secondary

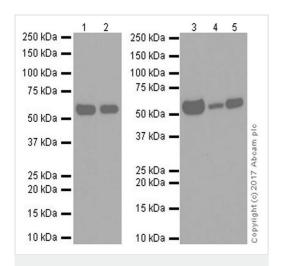
All lanes : Goat Anti-Rabbit $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$ at 1/100000 dilution

Predicted band size: 52 kDa

Observed band size: 52 kDa

Exposure time: 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Beclin 1 antibody [EPR20473] (ab210498)

All lanes : Anti-Beclin 1 antibody [EPR20473] (ab210498) at 1/1000 dilution

Lane 1 : Mouse brain lysate
Lane 2 : Mouse spleen lysate
Lane 3 : Rat brain lysate
Lane 4 : Rat kidney lysate
Lane 5 : Rat spleen lysate

Lysates/proteins at 10 µg per lane.

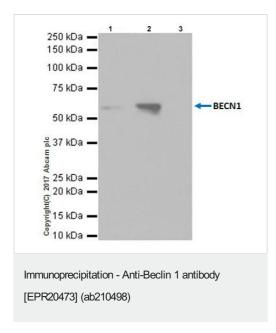
Secondary

All lanes : Goat Anti-Rabbit $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$ at 1/100000 dilution

Predicted band size: 52 kDa
Observed band size: 52 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Beclin 1 was immunoprecipitated from 0.35 mg of HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate with ab210498 at 1/30 dilution.

Western blot was performed from the immunoprecipitate using ab210498 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10000 dilution.

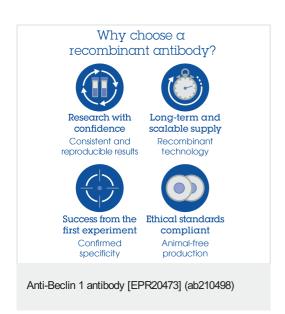
Lane 1: HepG2 whole cell lysate, 10 µg (Input).

Lane 2: ab210498 IP in HepG2 whole cell lysate.

Lane 3: Rabbit monoclonal $\lg G$ ($\underline{ab172730}$) instead of ab210498 in HepG2 whole cell lysate.

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

Exposure time: 30 seconds.



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