abcam

Product datasheet

Anti-Clathrin light chain antibody [EPR24231-72] ab271185



Recombinant

RabMAb

14 Images

Overview

Product name Anti-Clathrin light chain antibody [EPR24231-72]

DescriptionRabbit monoclonal [EPR24231-72] to Clathrin light chain

Host species Rabbit

Tested applications Suitable for: IHC-P, Flow Cyt (Intra), WB, IP, ICC/IF

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: HeLa, HEK-293, NCI-H1299, MEF, C2C12, PC-12 and C6 whole cell lysartes. Human

Clathrin light chain recombinant protein. IHC-P: Human cerebrum tissue; Mouse cerebrum tissue; Rat cerebrum tissue. ICC/IF: MEF, HeLa and C2C12 cells. Flow Cyt-intra: HeLa and C2C12

cells. IP: HeLa and MEF whole cell lysates.

General notesThis 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.

Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59.94% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

1

Clonality Monoclonal

Clone number EPR24231-72

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab271185 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
IHC-P		1/5000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/500.
WB		1/1000. Detects a band of approximately 35 kDa (predicted molecular weight: 27 kDa).
IP		1/30.
ICC/IF		1/250.

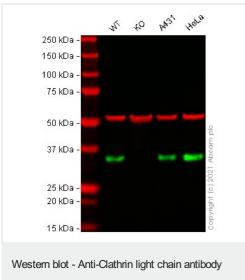
Target

Function Clathrin is the major protein of the polyhedral coat of coated pits and vesicles.

Cellular localization Cytoplasmic vesicle membrane. Membrane > coated pit. Cytoplasmic face of coated pits and

vesicles.

Images



[EPR24231-72] (ab271185)

All lanes : Anti-Clathrin light chain antibody [EPR24231-72] (ab271185) at 1/1000 dilution

Lane 1: Wild-type HEK-293T cell lysate

Lane 2: CLTA knockout HEK-293T cell lysate

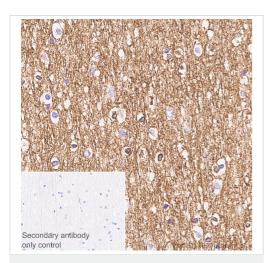
Lane 3 : A431 cell lysate
Lane 4 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 27 kDa **Observed band size:** 35 kDa

False colour image of Western blot: Anti-Clathrin light chain antibody [EPR24231-72] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab271185 was shown to bind specifically to Clathrin light chain. A band was observed at 35 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in CLTA knockout cell line ab267334 (knockout cell lysate ab258366). To generate this image, wild-type and CLTA knockout HEK-293T 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 (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.

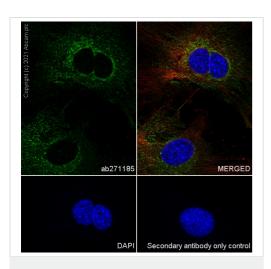


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Clathrin light chain antibody [EPR24231-72] (ab271185)

Immunohistochemical analysis of paraffin-embedded Human cerebrum tissue labelling Clathrin light chain with ab271185 at 1/5000 (0.102 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Cytoplasmic staining in human cerebrum. The section was incubated with ab271185 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 with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

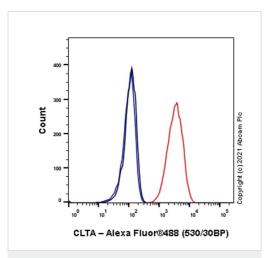


Immunocytochemistry/ Immunofluorescence - Anti-Clathrin light chain antibody [EPR24231-72] (ab271185)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized MEF cells labelling Clathrin light chain with ab271185 at 1/250 (2.036 ug/ml) dilution, followed by **ab150077** Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in MEF cells is observed. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor[®] 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

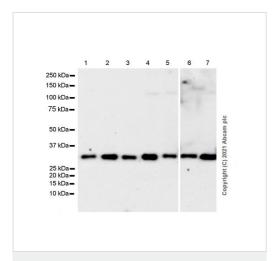
Secondary antibody only control: Secondary antibody is <u>ab150077</u>

Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-Clathrin light chain antibody [EPR24231-72] (ab271185)

Flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HeLa (Human cervix adenocarcinoma cell) cells labelling Clathrin light chain with ab271185 at 1/500 dilution (0.1ug)(Red) compared with a Rabbit monoclonal lgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit lgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.



Western blot - Anti-Clathrin light chain antibody [EPR24231-72] (ab271185)

All lanes : Anti-Clathrin light chain antibody [EPR24231-72] (ab271185) at 1/1000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 2: HEK-293 (human embryonic kidney epithelial cell) whole cell lysate

Lane 3: NCI-H1299 (human lung carcinoma epithelial cell) whole cell lysate

Lane 4 : MEF (mouse embryonic fibroblast (immortalized)) whole cell lysate

Lane 5 : C2C12 (mouse myoblasts myoblast) whole cell lysate

Lane 6 : PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate

Lane 7: C6 (rat glial tumor glial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

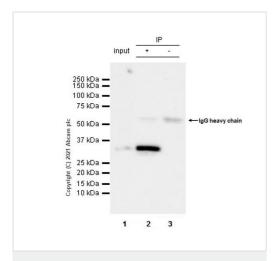
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/50000 dilution (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated)

Predicted band size: 27 kDa **Observed band size:** 35 kDa

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

The observed MW is consistent with what has been described in the literature (PMID: 31672988, 24852344).

Exposure time: 70 seconds.



Immunoprecipitation - Anti-Clathrin light chain antibody [EPR24231-72] (ab271185)

Clathrin light chain was immunoprecipitated from 0.35 mg HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate with ab271185 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab271185 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (ab131366) was used at 1/5000 dilution.

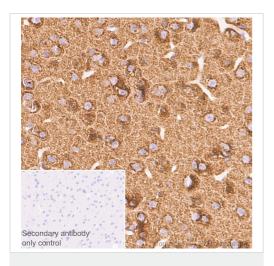
Lane 1: HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate 10 ug

Lane 2: ab271185 IP in HeLa whole cell lysate

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab271185 in HeLa whole cell lysate

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

Exposure time: 32 seconds

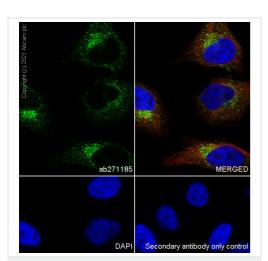


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Clathrin light chain antibody [EPR24231-72] (ab271185)

Immunohistochemical analysis of paraffin-embedded Mouse cerebrum tissue labelling Clathrin light chain with ab271185 at 1/5000 (0.102 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Cytoplasmic staining in mouse cerebrum. The section was incubated with ab271185 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).

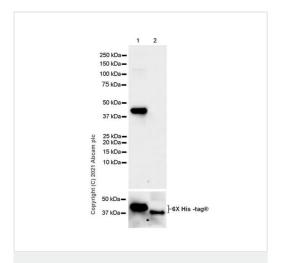
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunocytochemistry/ Immunofluorescence - Anti-Clathrin light chain antibody [EPR24231-72] (ab271185)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa cells labelling Clathrin light chain with ab271185 at 1/250 (2.036 ug/ml) dilution, followed by ab150077 Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in HeLa cells is observed. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor[®] 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) at 1/1000 dilution.



Western blot - Anti-Clathrin light chain antibody [EPR24231-72] (ab271185)

All lanes : Anti-Clathrin light chain antibody [EPR24231-72] (ab271185) at 1/1000 dilution

Lane 1: Human Clathrin light chain recombinant protein

Lane 2: Human CLTB recombinant protein

Lysates/proteins at 0.01 µg per lane.

Secondary

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

Predicted band size: 27 kDa **Observed band size:** 35 kDa

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

This antibody does not recognize human CLTB.

Exposure time: 70 seconds.

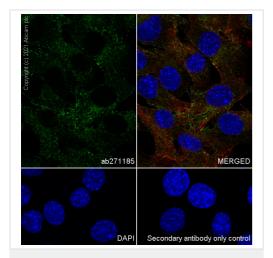


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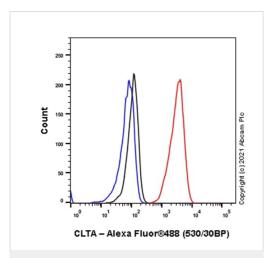
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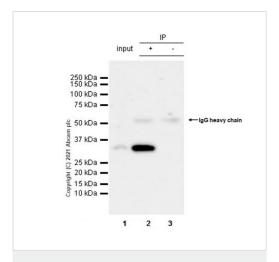
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized C2C12 cells labelling Clathrin light chain with ab271185 at 1/250 (2.036 ug/ml) dilution, followed by **ab150077** Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in C2C12 cells is observed. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor[®] 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

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Flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized C2C12 (Mouse myoblasts myoblast) cells labelling Clathrin light chain with ab271185 at 1/500 dilution (0.1ug) (Red) compared with a Rabbit monoclonal lgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit lgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.



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Clathrin light chain was immunoprecipitated from 0.35 mg MEF (mouse embryonic fibroblast (immortalized)) whole cell lysate with ab271185 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab271185 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (ab131366) was used at 1/5000 dilution.

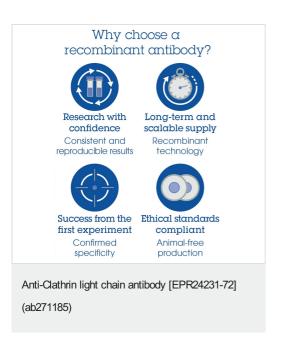
Lane 1: MEF (mouse embryonic fibroblast (immortalized)) whole cell lysate 10 ug

Lane 2: ab271185 IP in MEF whole cell lysate

Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab271185 in MEF whole cell lysate

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

Exposure time: 32 seconds



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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