abcam

Product datasheet

Anti-Clathrin heavy chain antibody [EPR24236-222] ab305259

Recombinant RabMAb

5 Images

Overview

Product name Anti-Clathrin heavy chain antibody [EPR24236-222]

Description Rabbit monoclonal [EPR24236-222] to Clathrin heavy chain

Host species Rabbit

Specificity The antibody detects both heavy chain 1 and 2.

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

Unsuitable for: IHC-P

Reacts with: Human Species reactivity

Does not react with: Mouse, Rat

Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. **Immunogen**

Positive control WB: HeLa and HEK-293T whole cell lysate. ICC/IF: HeLa cells. Flow Cyt (Intra): HeLa cells. IP:

HeLa 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: 7.20

Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS

Purity Protein A purified

Clonality Monoclonal

Clone number EPR24236-222

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab305259 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
WB		1/1000. Detects a band of approximately 180 kDa.
ICC/IF		1/50.
Flow Cyt (Intra)		1/500.
IP		1/30.

Application notes Is unsuitable for IHC-P.

Target

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

adapter protein complexes link the clathrin lattice either to the plasma membrane or to the trans-

Golgi network.

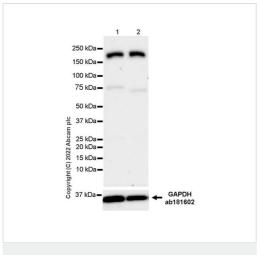
Sequence similarities Belongs to the clathrin heavy chain family.

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

coated pits and vesicles. Identified by mass spectrometry in melanosome fractions from stage I to

stage IV.

Images



Western blot - Anti-Clathrin heavy chain antibody [EPR24236-222] (ab305259)

All lanes : Anti-Clathrin heavy chain antibody [EPR24236-222] (ab305259) at 1/1000 dilution

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

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

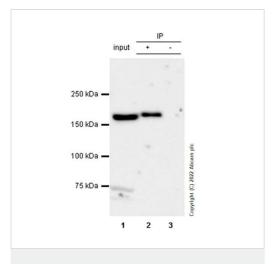
Lysates/proteins at 20 µg per lane.

Secondary

All lanes : VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) at 1/2000 dilution

Observed band size: 180 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST Exposure time: 37 seconds



Immunoprecipitation - Anti-Clathrin heavy chain antibody [EPR24236-222] (ab305259)

Clathrin heavy chain was immunoprecipitated from 0.35 mg HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg with ab305259 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab305259 at 1/1000 dilution. VeriBlot for IP secondary antibody (HRP) (ab131366) was used at 1/5000 dilution.

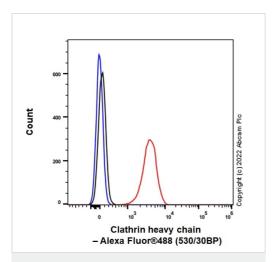
Lane 1: HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate 10 μg

Lane 2: ab305259 IP in HeLa whole cell lysate

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab305259 in HeLa whole cell lysate

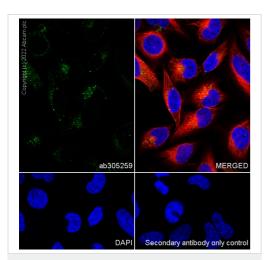
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 50 seconds



Flow Cytometry (Intracellular) - Anti-Clathrin heavy chain antibody [EPR24236-222] (ab305259)

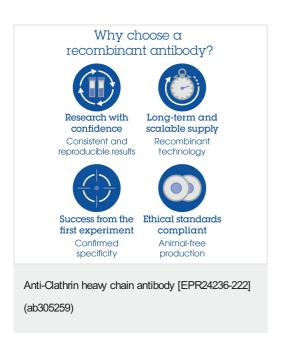
Flow cytometric (Intracellular) analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labelling Clathrin heavy chain with ab305259 at 1/500 dilution (0.1µg) (Red) compared with a Rabbit monoclonal lgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit lgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-Clathrin heavy chain antibody [EPR24236-222] (ab305259)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa (human cervical adenocarcinoma epithelial cell) cells labelling Clathrin heavy chain with ab305259 at 1/50 dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (Green). Confocal image showing punctuated cytoplasmic staining in HeLa cell line. The image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). is observed. ab195889 Antialpha 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 **ab150081**Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) preadsorbed at 1/1000 dilution.



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