abcam

Product datasheet

Anti-Clathrin heavy chain antibody [X22] ab2731

19 Abreviews 80 References 8 Images

Overview

Product name	Anti-Clathrin heavy chain antibody [X22]
Description	Mouse monoclonal [X22] to Clathrin heavy chain
Host species	Mouse
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Human, Xenopus laevis
Immunogen	Full length native protein (purified) corresponding to Human Clathrin heavy chain. Purified human brain clathrin heavy chain.
Epitope	Electron microscopy and proteolysis mapping demonstrate that binding occurs towards the central hub of the triskelion, N-terminal to the light chain binding regions.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.05% Sodium azide Constituent: PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	X22
Isotype	IgG1

Applications

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The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab2731 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	★★★☆☆ (5)	1/100 - 1/500. Detects a band of approximately 180 kDa.
IHC-P		1/100.
ICC/IF	★★★★★ (11)	1/1000.

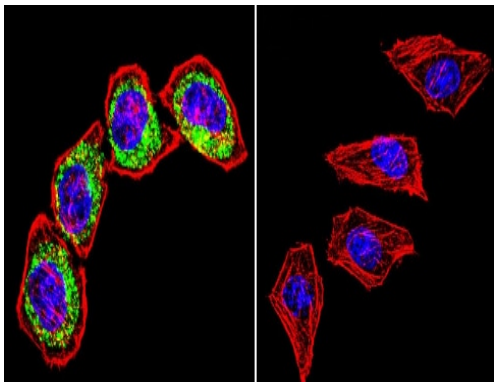
Target

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.

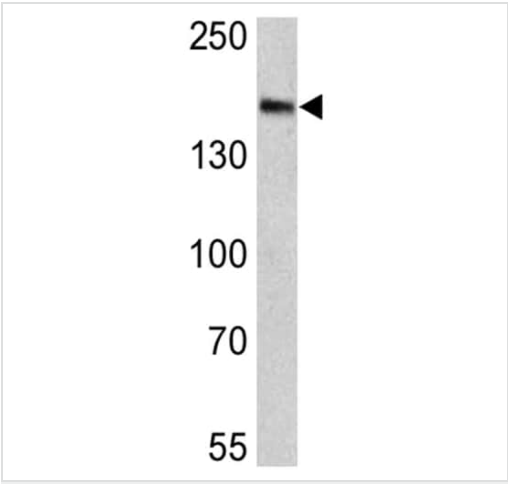
Cellular localization Cytoplasmic vesicle membrane. Membrane > coated pit. Melanosome. Cytoplasmic face of coated pits and vesicles. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

Images



Immunocytochemistry/ Immunofluorescence - Anti-Clathrin heavy chain antibody [X22] (ab2731)

Immunocytochemistry/Immunofluorescence analysis of Clathrin heavy chain shows staining in HeLa cells. Clathrin, Heavy chain staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with ab2731 (1:200) over night at 4°C, washed with PBS and incubated with a DyLight-488 conjugated goat anti-mouse secondary antibody. Images were taken at 60X magnification.



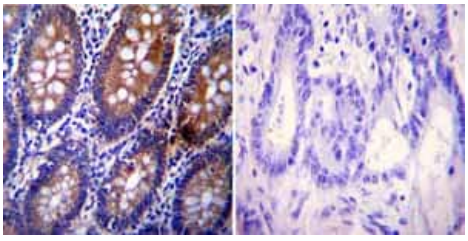
Western blot - Anti-Clathrin heavy chain antibody [X22] (ab2731)

Anti-Clathrin heavy chain antibody [X22] (ab2731) at 1/300 dilution + Human brain lysates at 25 µg

Secondary

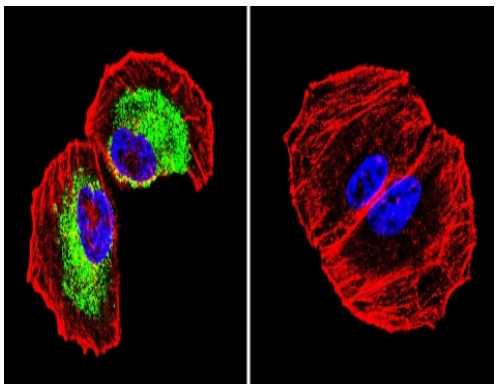
HRP-conjugated goat anti-mouse IgG + IgM (H+L)

Developed using the ECL technique.



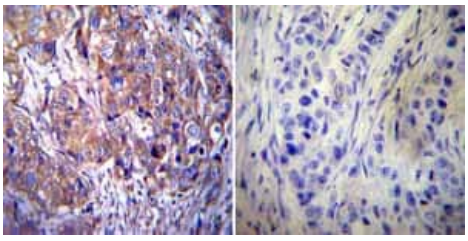
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Clathrin heavy chain antibody [X22] (ab2731)

Immunohistochemistry was performed on normal biopsies of deparaffinized Human colon tissue. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:100 with a mouse monoclonal antibody recognizing Clathrin Heavy chain ab2731 or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.



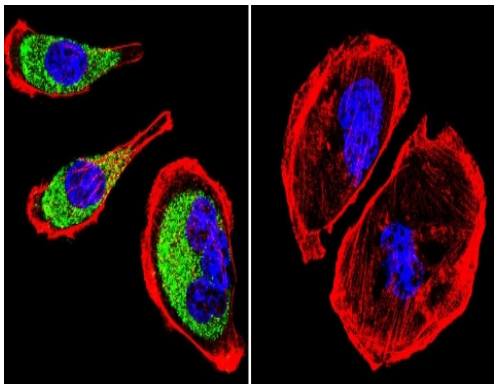
Immunocytochemistry/ Immunofluorescence - Anti-Clathrin heavy chain antibody [X22] (ab2731)

Immunocytochemistry/Immunofluorescence analysis of Clathrin heavy chain shows staining in NCI-H460 cells. Clathrin, Heavy chain staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with ab2731 (1:200) over night at 4°C, washed with PBS and incubated with a DyLight-488 conjugated goat anti-mouse secondary antibody. Images were taken at 60X magnification.



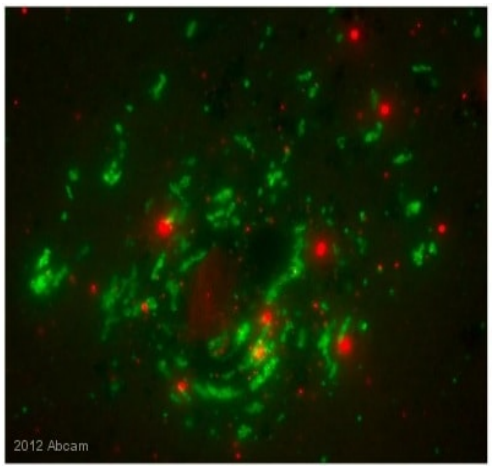
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Clathrin heavy chain antibody [X22] (ab2731)

Immunohistochemistry was performed on cancer biopsies of deparaffinized Human breast carcinoma tissue. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:100 with a mouse monoclonal antibody recognizing Clathrin Heavy chain ab2731 or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.



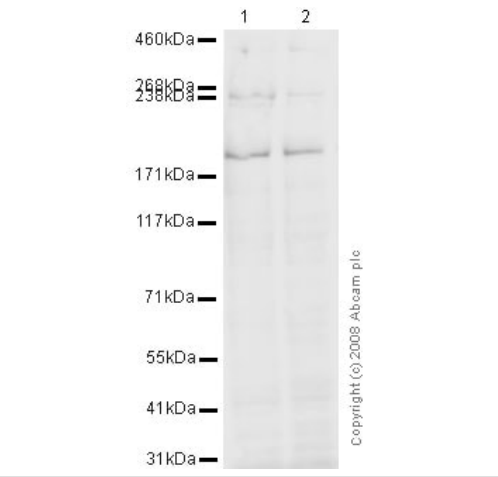
Immunocytochemistry/ Immunofluorescence - Anti-Clathrin heavy chain antibody [X22] (ab2731)

Immunocytochemistry/Immunofluorescence analysis of Clathrin heavy chain shows staining in U251 cells. Clathrin, Heavy chain staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with ab2731 (1:200) over night at 4°C, washed with PBS and incubated with a DyLight-488 conjugated goat anti-mouse secondary antibody. Images were taken at 60X magnification.



Immunocytochemistry/ Immunofluorescence - Anti-Clathrin heavy chain antibody [X22] (ab2731)

Xenopus laevis cytoplasmic egg extract visualized live with primary and secondary antibody addition [red is anti-Clathrin heavy chain X22 (ab2731) with goat anti-mouse Alexa Fluor 568 secondary, green is anti-HIP1R (**ab77297**) with goat anti-rabbit Alexa Fluor 488 secondary]. Large red structures are probably aggregates, but the small structures appear to be specific for vesicle staining.



Western blot - Anti-Clathrin heavy chain antibody [X22] (ab2731)

All lanes : Anti-Clathrin heavy chain antibody [X22] (ab2731) at 1/500 dilution

Lane 1 : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate
Lane 2 : Raji (Human Burkitt's lymphoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Observed band size: 180 kDa
Additional bands at: 240 kDa, 450 kDa. We are unsure as to the

identity of these extra bands.

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