Product datasheet

Anti-Clathrin heavy chain antibody [X22] ab2731

17 Abreviews  56 References  11 Images

Overview

Product name          Anti-Clathrin heavy chain antibody [X22]
Description           Mouse monoclonal [X22] to Clathrin heavy chain
Host species         Mouse
Specificity            Detects clathrin heavy chain.
Tested applications  Suitable for: ICC/IF, ICC, ELISA, Blocking, Flow Cyt, Immunomicroscopy, IP, WB, IHC-P
Species reactivity  Reacts with: Mouse, Rat, Cow, Dog, Human, Pig, Xenopus laevis, Bird, African green monkey
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.
Positive control      bovine brain extract

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

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.
**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.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICC/IF</td>
<td>★★★★★</td>
<td>1/1000.</td>
</tr>
<tr>
<td>ICC</td>
<td>★★★★★</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>ELISA</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>Blocking</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td>★★★★★</td>
<td>1/200. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>Immunomicroscopy</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>IP</td>
<td></td>
<td>Use at an assay dependent concentration. Immunoprecipitation of triskelions with this antibody allows both the clathrin heavy chain and the associated light chain polypeptides to be examined.</td>
</tr>
<tr>
<td>WB</td>
<td>★★★</td>
<td>1/500. Detects a band of approximately 180 kDa.</td>
</tr>
<tr>
<td>IHC-P</td>
<td></td>
<td>1/100.</td>
</tr>
</tbody>
</table>

**Target**

**Images**

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) 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 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.
Flow Cytometry - Anti-Clathrin heavy chain antibody [X22] (ab2731)

This image is courtesy of an Abreview submitted by Mahesh Shivananjappa

Platelets were isolated from Platelet rich plasma and suspended in PBS, fixed with paraformaldehyde and permeabilized with 0.1% Triton-X100 in 2% BSA for 30 minutes. The sample was incubated with the primary antibody (1/200 in PBS + 2% BSA) for 18 hours at 4°C. An Alexa Fluor®594-conjugated Goat anti-mouse IgG polyclonal (1/500) was used as the secondary antibody.

Gating Strategy: Platelets

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 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 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.

ab2731 staining Clathrin heavy chain - Membrane Vesicle Marker in Rat hypothalamus primary cells by ICC/IF (immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with 0.5% TX-100 and blocked with 5% serum for 20 minutes at 25°C. Samples were incubated with primary antibody (1/500 in 1% goat serum; 0.1% TX100; PBS) for 16 hours at 4°C. An Alexa Fluor®546-conjugated Goat polyclonal to mouse IgG, dilution 1/500, was used as secondary antibody.

ab2731 staining Clathrin heavy chain in pig retinal pigment epithelium (RPE) cells by Immunocytochemistry/Immunofluorescence. Cells were fixed with paraformaldehyde, permeabilized with 0.5% Triton ×100 and blocked with 5% serum for 20 minutes at 25°C. Samples were incubated with primary antibody (1/500: in 0.1% TX100, 1% goat serum, 1X PBS) for 16 hours at 4°C. An Alexa Fluor®546-conjugated goat polyclonal to mouse IgG was used as secondary antibody at 1/500 dilution.
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.

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

---

**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

why is the actual band size different from the predicted?

**Additional bands at**: 240 kDa, 450 kDa. We are unsure as to the identity of these extra bands.

---

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

**Our Abpromise to you: Quality guaranteed and expert technical support**

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
• Extensive multi-media technical resources to help you
• We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors