Product datasheet

Anti-Cytochrome C antibody [7H8.2C12] ab13575

Overview

Product name
Anti-Cytochrome C antibody [7H8.2C12]

Description
Mouse monoclonal [7H8.2C12] to Cytochrome C

Host species
Mouse

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

Species reactivity
Reacts with: Human

Predicted to work with: Mouse, Rat, Horse, Pigeon, Drosophila melanogaster

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

Epitope
The antibody recognizes an epitope within amino acids 93-104 of pigeon Cytochrome C, based on competitive ELISA results.

Positive control
WB: HeLa, Jurkat and human heart whole cell lysates; IHC-P: Human liver and skin tissues; ICC/IF: Leukocytes from murine bone marrow; Flow Cyt (Intra): HepG2 cells.

General notes
This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com.

The Life Science industry has been in the grip 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
pH: 7.40
Preservative: 0.02% Sodium azide
Constituents: PBS, 6.97% L-Arginine

Purity
Protein G purified
Clonality: Monoclonal
Clone number: 7H8.2C12
Isotype: IgG2b
Light chain type: kappa

Applications

The Abpromise guarantee: Our Abpromise guarantee covers the use of ab13575 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐⭐️ (20)</td>
<td>Use a concentration of 1 - 5 µg/ml. Detects a band of approximately 15 kDa (predicted molecular weight: 12 kDa).</td>
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<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐⭐️ (2)</td>
<td>Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
</tr>
<tr>
<td>Flow Cyt (Intra)</td>
<td></td>
<td>Use 0.1-1µg for 10^6 cells. <strong>ab170192</strong> - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐⭐️ (6)</td>
<td>Use at an assay dependent concentration.</td>
</tr>
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Target

Function: Electron carrier protein. The oxidized form of the cytochrome c heme group can accept an electron from the heme group of the cytochrome c1 subunit of cytochrome reductase. Cytochrome c then transfers this electron to the cytochrome oxidase complex, the final protein carrier in the mitochondrial electron-transport chain.

Plays a role in apoptosis. Suppression of the anti-apoptotic members or activation of the pro-apoptotic members of the Bcl-2 family leads to altered mitochondrial membrane permeability resulting in release of cytochrome c into the cytosol. Binding of cytochrome c to Apaf-1 triggers the activation of caspase-9, which then accelerates apoptosis by activating other caspases.

Involvement in disease: Defects in CYCS are the cause of thrombocytopenia type 4 (THC4) [MIM:612004]; also known as autosomal dominant thrombocytopenia type 4. Thrombocytopenia is the presence of relatively few platelets in blood. THC4 is a non-syndromic form of thrombocytopenia. Clinical manifestations of thrombocytopenia are absent or mild. THC4 may be caused by dysregulated platelet formation.

Sequence similarities: Belongs to the cytochrome c family.

Post-translational modifications: Binds 1 heme group per subunit.

Cellular localization: Mitochondrion matrix.

Images
Immunocytochemistry/ Immunofluorescence - Anti-Cytochrome C antibody [7H8.2C12] (ab13575)

ab13575 staining Cytochrome C in HepG2 cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab13575 at 1µg/ml and ab6046. Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with ab150117, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and ab150080, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour magenta). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.

Western blot - Anti-Cytochrome C antibody [7H8.2C12] (ab13575)

All lanes : Anti-Cytochrome C antibody [7H8.2C12] (ab13575) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate
Lane 3 : Human heart tissue lysate - total protein (ab29431)

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Mouse IgG H&L (HRP) preadsorbed (ab97040) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 12 kDa

Additional bands at: 14 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 3 minutes
Abcam recommends using milk (5%) as the blocking agent. Abcam welcomes customer feedback and would appreciate any comments regarding this product and the data presented above.

**Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytochrome C antibody [7H8.2C12] (ab13575)**

IHC image of Cytochrome C staining in human normal liver formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab13575, 1µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

**Overlay histogram showing HepG2 cells stained with ab13575 (red line).** The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab13575, 0.1µg/1x10⁶ cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1]/mouse IgG2b [PLPV219] (ab91353/ab91366, 1µg/1x10⁶ cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.
Ab13575 staining human normal skin tissue. Staining is localised to mitochondria.
Left panel: with primary antibody at 4 ug/ml. Right panel: isotype control.
Sections were stained using an automated system DAKO Autostainer Plus, at room temperature. Sections were rehydrated and antigen retrieved with the Dako 3-in-1 antigen retrieval buffer EDTA pH 9.0 in a DAKO PT Link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 minutes. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 minutes and detected with Dako Envision Flex amplification kit for 30 minutes. Colorimetric detection was completed with diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that for manual staining we recommend to optimize the primary antibody concentration and incubation time (overnight incubation), and amplification may be required.

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

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