Product datasheet

Anti-ATP citrate lyase antibody [EP704Y] ab40793

Overview

Product name
Anti-ATP citrate lyase antibody [EP704Y]

Description
Rabbit monoclonal [EP704Y] to ATP citrate lyase

Host species
Rabbit

Specificity
ab40793 recognise ATP citrate lyase (ACL). The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.

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

Species reactivity
Reacts with: Mouse, Rat, Human, Common marmoset

Immunogen
Synthetic peptide within Human ATP citrate lyase aa 1050 to the C-terminus (C terminal). The exact sequence is proprietary.

(Peptide available as ab207504)

Positive control
WB: HeLa cell lysate; NIH/3T3; rat lung; C6 lysates. ICC/IF: HeLa cells. Flow Cyt: HeLa cells. IP: Jurkat cell lysate; HeLa. IHC: Human clear cell carcinoma of kidney

General notes
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

This product is a recombinant rabbit monoclonal antibody.

Properties

Form
Liquid

Storage instructions

Storage buffer
pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: 40% Glycerol, 0.05% BSA, 59% PBS

Purity
Protein A purified
Clonality: Monoclonal
Clone number: EP704Y
Isotype: IgG

Applications

Our Abpromise guarantee covers the use of ab40793 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
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<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>ICC/IF</td>
<td>1/50.</td>
<td></td>
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<tr>
<td>IHC-P</td>
<td>1/100. PubMed: 19727777 The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.</td>
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<tr>
<td>WB</td>
<td><img src="https://www.abcam.com/images/ab161790_m.png" alt="" /> 1/10000. Detects a band of approximately 125 kDa (predicted molecular weight: 122 kDa). For unpurified use at 1/1000 - 1/5000.</td>
<td></td>
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<tr>
<td>Flow Cyt</td>
<td>1/30. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For unpurified use at 1/100.</td>
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<tr>
<td>IP</td>
<td>1/20.</td>
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Target

Function: ATP-citrate synthase is the primary enzyme responsible for the synthesis of cytosolic acetyl-CoA in many tissues. Has a central role in de novo lipid synthesis. In nervous tissue it may be involved in the biosynthesis of acetylcholine.

Sequence similarities:
- In the N-terminal section; belongs to the succinate/malate CoA ligase beta subunit family.
- In the C-terminal section; belongs to the succinate/malate CoA ligase alpha subunit family.
- Contains 1 ATP-grasp domain.

Post-translational modifications:
- ISGylated.
- Acetylated at Lys-540, Lys-546 and Lys-554 by KAT2B/PCAF. Acetylation is promoted by glucose and stabilizes the protein, probably by preventing ubiquitination at the same sites. Acetylation promotes de novo lipid synthesis. Deacetylated by SIRT2.
- Ubiquitinated at Lys-540, Lys-546 and Lys-554 by UBR4, leading to its degradation. Ubiquitination is probably inhibited by acetylation at same site.

Cellular localization: Cytoplasm.

Images
All lanes: Anti-ATP citrate lyase antibody [EP704Y] (ab40793) at 1/10000 dilution (purified)

Lane 1: Rat lung lysates
Lane 2: C6 (Rat glial tumor glial cell) whole cell lysate

Lysates/proteins at 15 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 122 kDa

Blocking and diluting buffer: 5% NFDM/TBST.

All lanes: Anti-ATP citrate lyase antibody [EP704Y] (ab40793) at 1/50000 dilution (purified)

Lane 1: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates
Lane 2: NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates

Lysates/proteins at 20 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 122 kDa

Blocking and diluting buffer: 5% NFDM/TBST.
Immunoprecipitation - Anti-ATP citrate lyase antibody [EP704Y] (ab40793)

ab40793 (purified) at 1:20 dilution (1.5μg) immunoprecipitating ATP citrate lyase in HeLa whole cell lysate.

**Lane 1 (input):** HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate, 10μg  
**Lane 2 (+):** ab40793 & HeLa whole cell lysate  
**Lane 3 (-):** Rabbit monoclonal IgG (ab172730) instead of ab40793 in HeLa whole cell lysate.

For western blotting, VeriBlot for IP secondary antibody (HRP) (ab131366) was used as the secondary antibody at 1:1000 dilution. Blocking and diluting buffer: 5% NFDM/TBST.

Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling ATP citrate lyase with purified ab40793 at 1:30 dilution (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1:2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human clear cell carcinoma of kidney tissue sections labeling ATP citrate lyase with Purified ab40793 at 1:100 dilution. Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)
Lane 2: ATP citrate lyase knockout HAP1 whole cell lysate (20 µg)
Lane 3: HeLa whole cell lysate (20 µg)
Lanes 1 - 3: Merged signal (red and green). Green - ab40793 observed at 125 kDa. Red - loading control, ab9484, observed at 37 kDa.

Unpurified ab40793 was shown to specifically react with ATP citrate lyase in wild-type HAP1 cells as signal was lost in ATP citrate lyase knockout cells. Wild-type and ATP citrate lyase knockout samples were subjected to SDS-PAGE. ab40793 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.
Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling ATP citrate lyase with purified ab40793 at 1/50. Cells were fixed with 100% methanol. *ab150077*, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody.

Control: PBS only.

Nuclear counter stain: DAPI.

Anti-ATP citrate lyase antibody [EP704Y] (ab40793) at 1/5000 dilution (unpurified) + HeLa cell lysate

**Predicted band size:** 122 kDa

**Observed band size:** 122 kDa

Overlay histogram showing HeLa cells stained with unpurified ab40793 (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 (ab40793, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1μg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.
Unpurified ab40793 at 1/40 immunoprecipitating ATP citrate lyase in HeLa (human cervix adenocarcinoma) whole cell lysate.

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

Lane 2 (+): ab40793 + HeLa (human cervix adenocarcinoma) whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab40793 in HeLa (human cervix adenocarcinoma) whole cell lysate

For western blotting, ab40793 at 1/1000 dilution and ab131366 VeriBlot for IP (HRP) was used as the secondary antibody at 1/10000.

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

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