abcam

Product datasheet

Anti-ATP citrate lyase antibody [EP704Y] ab40793





★★★★★ 2 Abreviews 52 References 11 Images

Overview

Product name Anti-ATP citrate lyase antibody [EP704Y]

Description Rabbit monoclonal [EP704Y] to ATP citrate lyase

Host species Rabbit

Specificity This antibody recognises 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: IHC-P, Flow Cyt (Intra), ICC/IF, WB, IP

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Common marmoset

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

(Peptide available as ab207504)

Positive control WB: HeLa cell lysate; NIH/3T3; rat lung; C6 lysates. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa

cells. IP: Jurkat cell lysate; HeLa. IHC: Human clear cell carcinoma of kidney

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

Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long Storage instructions

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

ClonalityMonoclonalClone numberEP704Y

Isotype IgG

Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> 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.

Application	Abreviews	Notes
IHC-P		Use at an assay dependent concentration.
Flow Cyt (Intra)		1/30. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody. For unpurified use at 1/100.
ICC/IF		1/50.
WB	★★★★☆ (2)	1/10000. Detects a band of approximately 125 kDa (predicted molecular weight: 122 kDa). Can be blocked with ATP citrate lyase peptide (ab207504). For unpurified use at 1/1000 - 1/5000.
IP		1/20.

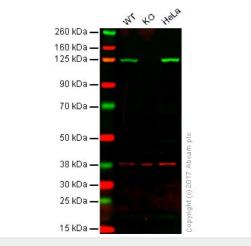
Function	ATP-citrate synthase is the primary enzyme responsible for the synthesis of cytosolic acetyl-CoA in many tissues. Has a central role in de novo lipid synthesis. In nervous tissue it may be involved in the biosynthesis of acetylcholine.
Sequence similarities	In the N-terminal section; belongs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

Target



Western blot - Anti-ATP citrate lyase antibody [EP704Y] (ab40793)

Immunocytochemistry/ Immunofluorescence - Anti-ATP citrate lyase antibody [EP704Y] (ab40793)

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)

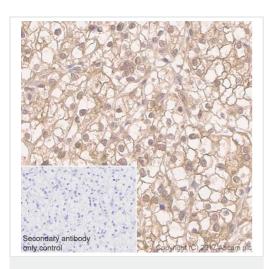
Lanes 1 - 3: Merged signal (red and green). Green - ab40793 observed at 125 kDa. Red - loading control, ab9484, observed at 37 kDa.

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 lgG 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® 488conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody.

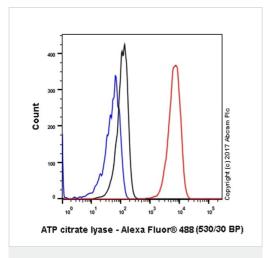
Control: PBS only.

Nuclear counter stain: DAPI.



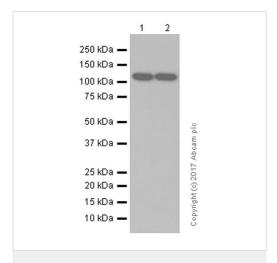
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ATP citrate lyase antibody [EP704Y] (ab40793)

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.

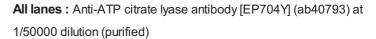


Flow Cytometry (Intracellular) - Anti-ATP citrate lyase antibody [EP704Y] (ab40793)

Intracellular 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 lgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal lgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Western blot - Anti-ATP citrate lyase antibody [EP704Y] (ab40793)



Lane 1: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

Lane 2: NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates

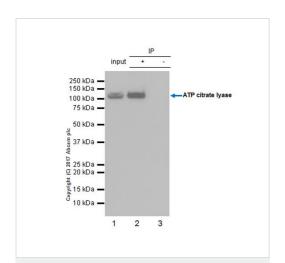
Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) 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.

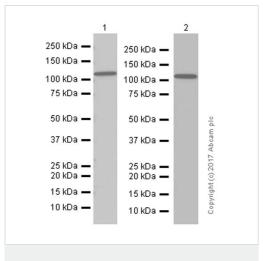
Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate,10µg

Lane 2 (+): ab40793 & HeLa whole cell lysate

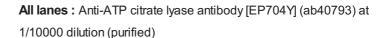
Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab40793 in HeLa whole cell lysate.

For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1:1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.



Western blot - Anti-ATP citrate lyase antibody [EP704Y] (ab40793)



Lane 1: Rat lung lysates

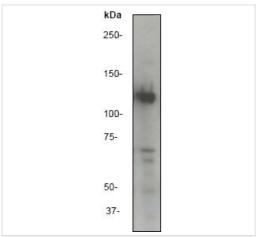
Lane 2: C6 (Rat glial tumor glial cell) whole cell lysate

Lysates/proteins at 15 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 122 kDa

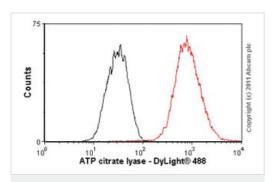


Western blot - Anti-ATP citrate lyase antibody [EP704Y] (ab40793)

Blocking and diluting buffer: 5% NFDM/TBST.

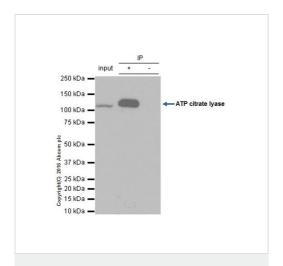
Anti-ATP citrate lyase antibody [EP704Y] (ab40793) at 1/5000 dilution (unpurified) + HeLa cell lysate

Predicted band size: 122 kDa **Observed band size:** 122 kDa



Flow Cytometry (Intracellular) - Anti-ATP citrate lyase antibody [EP704Y] (ab40793)

Overlay histogram showing HeLa cells stained withunpurified 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 lgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) ($l\mu g/1x10^6$ cells) used under the same conditions. Acquisition of >5,000 events was performed.



Immunoprecipitation - Anti-ATP citrate lyase antibody [EP704Y] (ab40793)

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 lgG (<u>ab172730</u>) 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 for detection at 1/10000.

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



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