abcam

Product datasheet

Anti-CPT1A antibody [EPR21843-71-1C] ab220789

KO VALIDATED Recombinant RabMAb

5 References 12 Images

Overview

Product name	Anti-CPT1A antibody [EPR21843-71-1C]
Description	Rabbit monoclonal [EPR21843-71-1C] to CPT1A
Host species	Rabbit
Specificity	Our WB images were generated by testing un-bolied samples.
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Wild-type HAP1 whole cell lysate; HEK-293T, HeLa, SK-OV-3, MCF7 and HepG2 whole cell lysates; Human kidney lysate; His-tagged human CPT1A recombinant protein (aa406-755). IHC-P: Human kidney and ovarian carcinoma tissues. ICC/IF: HeLa and SK-OV-3 cells. Flow Cyt (intra): HeLa cells. IP: SK-OV-3 whole cell lysate.
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
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR21843-71-1C
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab220789 in the following tested applications.

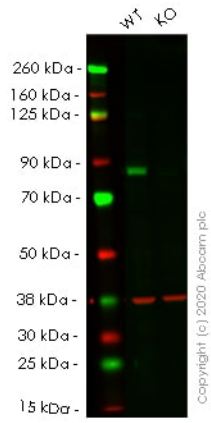
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/600.
WB		1/1000. Detects a band of approximately 88 kDa (predicted molecular weight: 88 kDa). Our WB images were generated by testing un-bolied samples.
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/100.
IP		1/30.

Target

Tissue specificity	Strong expression in kidney and heart, and lower in liver and skeletal muscle.
Pathway	Lipid metabolism; fatty acid beta-oxidation.
Involvement in disease	Defects in CPT1A are the cause of carnitine palmitoyltransferase 1A deficiency (CPT1AD) [MIM:255120]; also known as CPT-I deficiency or CPT1A deficiency. CPT1AD is a rare autosomal recessive metabolic disorder of long-chain fatty acid oxidation characterized by severe episodes of hypoketotic hypoglycemia usually occurring after fasting or illness. Onset is in infancy or early childhood.
Sequence similarities	Belongs to the carnitine/choline acetyltransferase family.
Cellular localization	Mitochondrion outer membrane.

Images



Western blot - Anti-CPT1A antibody [EPR21843-71-1C] (ab220789)

All lanes : Anti-CPT1A antibody [EPR21843-71-1C] (ab220789) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : CPT1A knockout HEK-293T cell lysate

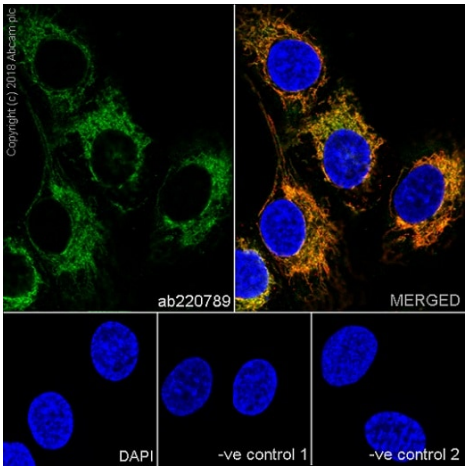
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 88 kDa
Observed band size: 88 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab220789 observed at 88 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab220789 was shown to react with CPT1A in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab266319** (knockout cell lysate **ab256880**) was used. Wild-type HEK-293T and CPT1A knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab220789 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-CPT1A antibody [EPR21843-71-1C] (ab220789)

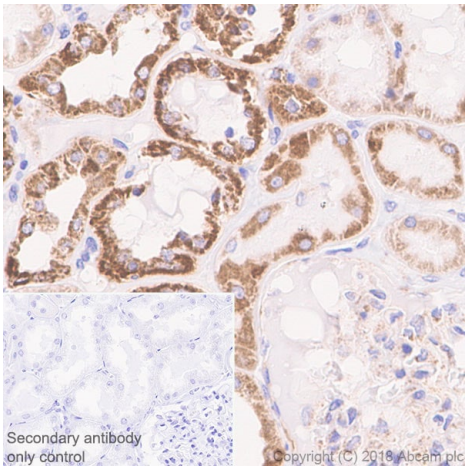
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized SK-OV-3 (human ovarian cancer cell line) cells labeling CPT1A with ab220789 at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing mitochondrial staining in SK-OV-3 cell line.

The nuclear counter stain is DAPI (blue). COX IV is detected with Anti-COX IV antibody [mAbcam33985] - Mitochondrial Marker (**ab33985**) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150120**) (red).

The negative controls are as follows:

-ve control 1: ab220789 at 1/100 dilution followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150120**) secondary antibody at 1/1000 dilution.

-ve control 2: Anti-COX IV antibody [mAbcam33985] - Mitochondrial Marker (**ab33985**) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**).

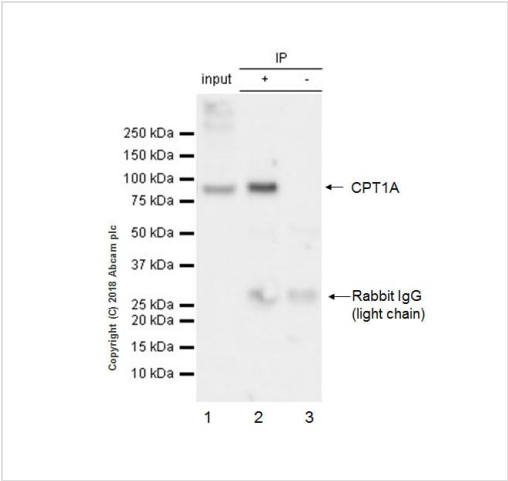


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CPT1A antibody [EPR21843-71-1C] (ab220789)

Immunohistochemical analysis of paraffin-embedded human kidney tissue labeling CPT1A with ab220789 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Granular cytoplasmic staining in human kidney (PMID: 18192268; PMID: 28956034). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-CPT1A antibody [EPR21843-71-1C] (ab220789)

CPT1A was immunoprecipitated from 0.35 mg of SK-OV-3 (human ovarian cancer cell line) whole cell lysate with ab220789 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab220789 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/5000 dilution.

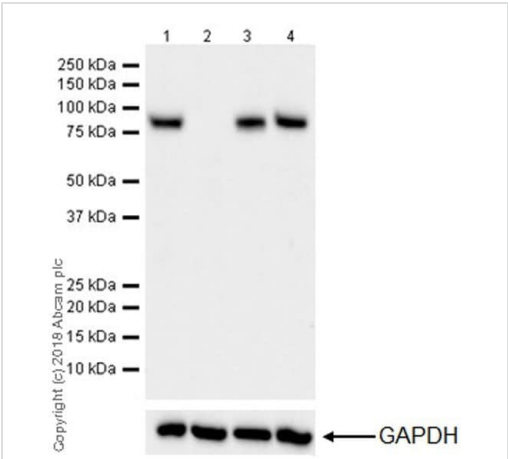
Lane 1: SK-OV-3 whole cell lysate 10 µg (Input).

Lane 2: ab220789 IP in SK-OV-3 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab220789 in SK-OV-3 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 30 seconds.



Western blot - Anti-CPT1A antibody [EPR21843-71-1C] (ab220789)

All lanes : Anti-CPT1A antibody [EPR21843-71-1C] (ab220789) at 1/5000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : CPT1A knockout HAP1 whole cell lysate
Lane 3 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 4 : SK-OV-3 (human ovarian cancer cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

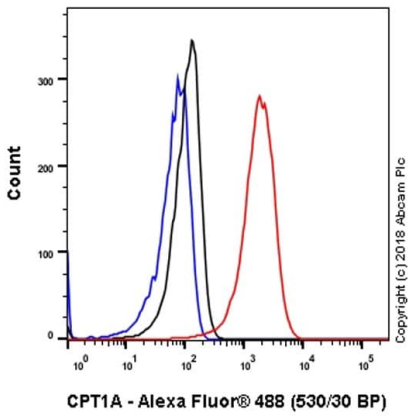
Developed using the ECL technique.

Predicted band size: 88 kDa
Observed band size: 88 kDa

Exposure time: 92 seconds

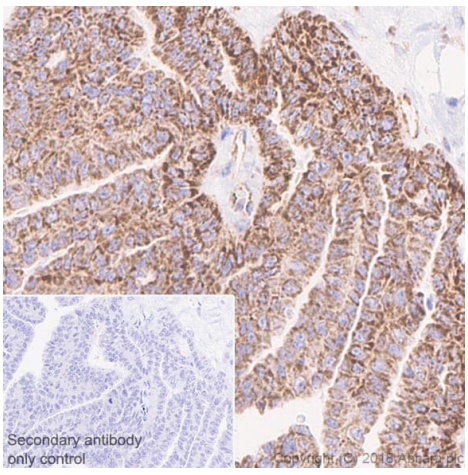
Blocking/Dilution buffer: 5% NFDM/TBST.

ab220789 was shown to specifically react with CPT1A in wild-type HAP1 cells as signal was lost in CPT1A knockout cells. Wild-type and CPT1A knockout samples were subjected to SDS-PAGE. ab220789 and **ab181602** (Rabbit anti-GAPDH loading control) were incubated 1 hour at room temperature at 1/5000 dilution and 1/200,000 dilution respectively. Blots were developed with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) secondary antibody at 1/100,000 dilution for 1 hour at room temperature before imaging. The blot was developed on a BIO-RAD® ChemiDoc™ MP instrument using the ECL technique.



Flow Cytometry (Intracellular) - Anti-CPT1A antibody [EPR21843-71-1C] (ab220789)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cell line labeling CPT1A with ab220789 at 1/600 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/2000 dilution was used as the secondary antibody.

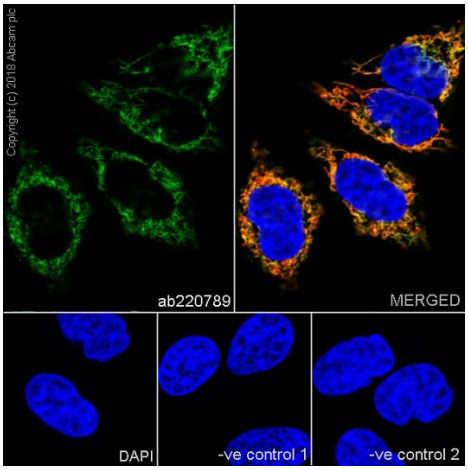


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CPT1A antibody [EPR21843-71-1C] (ab220789)

Immunohistochemical analysis of paraffin-embedded human ovarian carcinoma tissue labeling CPT1A with ab220789 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Granular cytoplasmic staining in human ovarian carcinoma (PMID: 26716645). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-CPT1A antibody [EPR21843-71-1C] (ab220789)

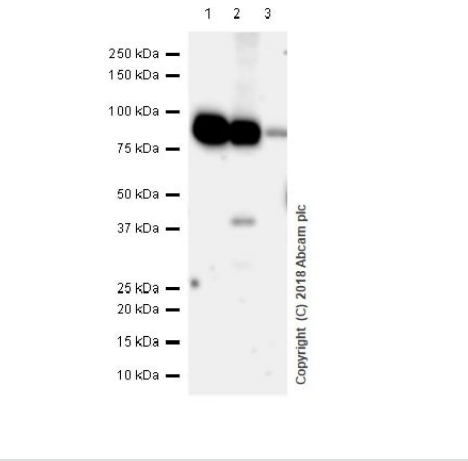
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling CPT1A with ab220789 at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing mitochondrial staining in HeLa cell line.

The nuclear counter stain is DAPI (blue). COX IV is detected with Anti-COX IV antibody [mAbcam33985] - Mitochondrial Marker (**ab33985**) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150120**) (red).

The negative controls are as follows:

-ve control 1: ab220789 at 1/100 dilution followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150120**) secondary antibody at 1/1000 dilution.

-ve control 2: Anti-COX IV antibody [mAbcam33985] - Mitochondrial Marker (**ab33985**) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**).



Western blot - Anti-CPT1A antibody [EPR21843-71-1C] (ab220789)

All lanes : Anti-CPT1A antibody [EPR21843-71-1C] (ab220789) at 1/1000 dilution

Lane 1 : MCF7 (Human breast adenocarcinoma epithelial cell) cell lysate
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) cell lysate
Lane 3 : HepG2 (Human hepatocellular carcinoma epithelial cell) cell lysate

Lysates/proteins at 20 µg per lane.

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

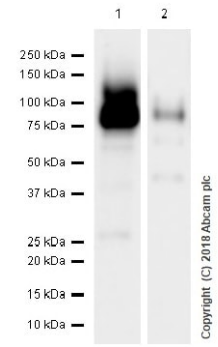
Predicted band size: 88 kDa
Observed band size: 88 kDa

Exposure time: 180 seconds

Blocking and diluting buffer and concentration: 5% NFDM /TBST.

CPT1A is strongly expressed in kidney and heart, and lower in liver and skeletal muscle.

We recommend loading higher amount of lysate or using lower antibody dilution to detect signal in HepG2 lysate.



Western blot - Anti-CPT1A antibody [EPR21843-71-1C] (ab220789)

All lanes : Anti-CPT1A antibody [EPR21843-71-1C] (ab220789) at 1/1000 dilution

Lane 1 : Human kidney tissue lysate
Lane 2 : Human liver tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : VeriBlot for IP Detection Reagent (HRP) (**ab131366**) at 1/1000 dilution

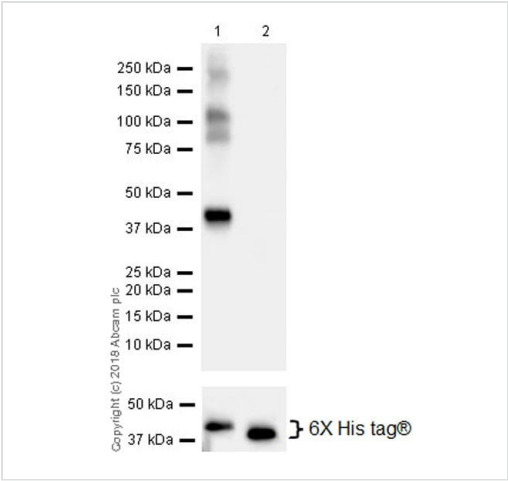
Predicted band size: 88 kDa
Observed band size: 88 kDa

Exposure time: 92 seconds

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

CPT1A is strongly expressed in kidney and heart, and lower in liver and skeletal muscle.

We recommend loading higher amount of lysate or using lower antibody dilution to detect signal in liver lysate.



Western blot - Anti-CPT1A antibody [EPR21843-71-1C] (ab220789)

All lanes : Anti-CPT1A antibody [EPR21843-71-1C] (ab220789) at 1/1000 dilution

Lane 1 : His-tagged human CPT1A recombinant protein (aa406-755)
Lane 2 : His-tagged human CPT1B recombinant protein (aa407-756)

Lysates/proteins at 0.02 µg per lane.

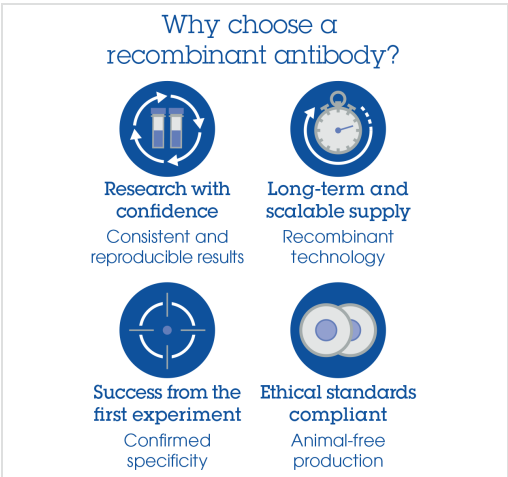
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 88 kDa
Observed band size: 38 kDa

Exposure time: 10 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Anti-CPT1A antibody [EPR21843-71-1C] (ab220789)

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