abcam

Product datasheet

Anti-CPT1A antibody [8F6AE9] ab128568

KO VALIDATED

4 Abreviews 41 References 7 Images

Overview

Product name	Anti-CPT1A antibody [8F6AE9]
Description	Mouse monoclonal [8F6AE9] to CPT1A
Host species	Mouse
Tested applications	**Suitable for:** Flow Cyt, IHC-P, WB, ICC/IF, In-Cell ELISA
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment, corresponding to amino acids 489-773 of Human CPT1A.
Positive control	Partial Human Recombinant CPT1A protein; HepG2, H9C2, H4IIE Whole Cell Lysates; Rat heart mitochondrial, Human liver, Rat liver and Mouse liver homogenates; H9C2 cells; HeLa cells IHC-P: human normal kidney FFPE tissue sections
General notes	This antibody clone is manufactured by Abcam. This monoclonal antibody to CPT1A has been knockout validated in Western blot. The expected band for CPT1A was observed in wild type cells and the band was not seen in CPT1A knockout cells. If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com or you can find further information here.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	Preservative: 0.02% Sodium azide Constituent: HEPES buffered saline
Purity	Ammonium Sulphate Precipitation
Purification notes	Purity is near homogeneity as judged by SDS-PAGE. The antibody was produced in vitro using hybridomas grown in serum-free medium, and then concentrated by ammonium sulfate precipitation.
Clonality	Monoclonal
Clone number	8F6AE9

Isotype	IgG2b
Light chain type	kappa

Applications

Our Abpromise guarantee covers the use of **ab128568** in the following tested applications.

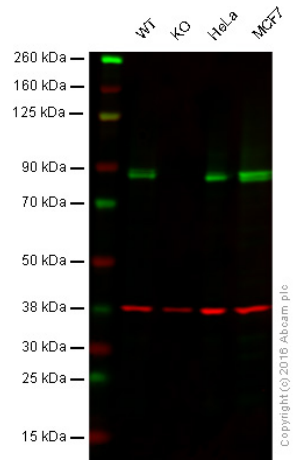
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt		Use at an assay dependent concentration.
IHC-P		Use a concentration of 0.5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB	★★★★★	Use a concentration of 1 µg/ml. Predicted molecular weight: 88 kDa.
ICC/IF	★★★★★	Use a concentration of 0.5 - 5 µg/ml.
In-Cell ELISA		Use a concentration of 0.1 - 1 µg/ml.

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

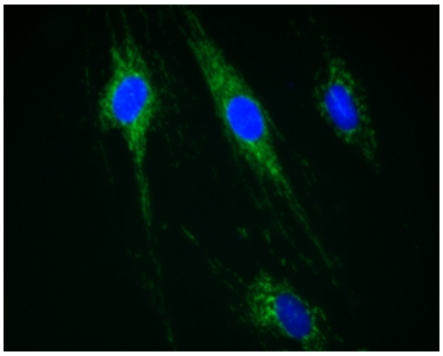


Western blot - Anti-CPT1A antibody [8F6AE9] (ab128568)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: CPT1A knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: MCF-7 cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab128568 observed at 88 kDa. Red - loading control, ab181602, observed at 37 kDa.

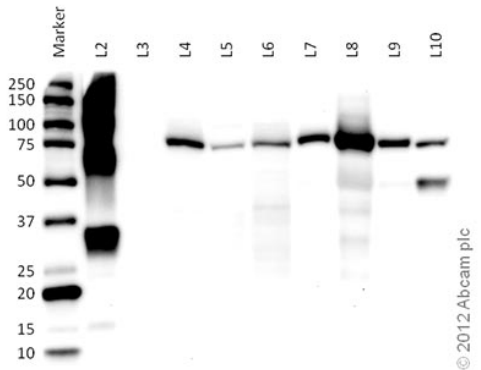
ab128568 specifically detected the expected band for CPT1A in wild-type HAP1 cells. No band was observed in CPT1A knockout cells. Wild-type and CPT1A knockout samples were subjected to SDS-PAGE. ab128568 and ab181602 (loading control to GAPDH) were diluted at 1 µg/mL and 1/10000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (ab216772) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-CPT1A antibody [8F6AE9] (ab128568)

Immunofluorescent staining of CPT1A in H9C2 cells (rat) using ab128568.
Fixation: 4% paraformaldehyde PBS fixed for 20 minutes
Permebilization: 0.1% Triton X-100 PBS for 30 minutes at room temperature while rocking
Blocking: 2x Sigma Block 0.1% Triton X-100 H2O for 2 hours at room temperature while rocking
Primary antibodies: Anti-CPT1A antibody (ab128568) 0.5 ug/mL 1x Sigma Block with 0.1% Triton X-100 incubated overnight at 4 °C.
Washing: 3x 1% NGS 10 minutes/wash.
Secondary antibodies: Alexa 488 GAM 1:1000 diluted in 1% NGS with 0.1% Triton X-100 PBS incubated for 2 hours at room temperature while rocking.
Washing: 3x 1% NGS 10 minutes/wash.
DAPI: 20 ng/mL in 1% NGS, 0.1% Triton X-100 PBS.

Washing: 1x 1% NGS 10 minutes/wash.



Western blot - Anti-CPT1A antibody [8F6AE9] (ab128568)

All lanes : Anti-CPT1A antibody [8F6AE9] (ab128568) at 1 µg/ml

Lane 1 : Marker
Lane 2 : Partial Human Recombinant CPT1A protein (ab128569) at 0.1 µg
Lane 3 : Full Length Human Recombinant OTC protein at 0.1 µg
Lane 4 : HepG2 (Human hepatocellular carcinoma cell line) Whole Cell Lysate at 20 µg
Lane 5 : H9C2 (Rat cardiomyoblast cell line) Whole Cell Lysate at 20 µg
Lane 6 : H4IIE (Rat hepatoma cell line) Whole Cell Lysate at 20 µg
Lane 7 : RHM (Rat heart mitochondrial homogenate) at 20 µg
Lane 8 : HLH (Human liver homogenate) at 20 µg
Lane 9 : RLH (Rat liver homogenate) at 20 µg
Lane 10 : MLH (Mouse liver homogenate) at 20 µg

Secondary
All lanes : Goat polyclonal to Mouse IgG – H&L – Pre-Adsorbed (HRP) at 1/10000 dilution

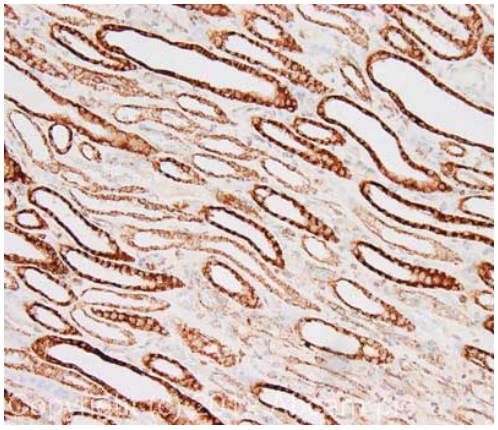
Developed using the ECL technique.

Performed under reducing conditions.

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

Exposure time: 1 minute

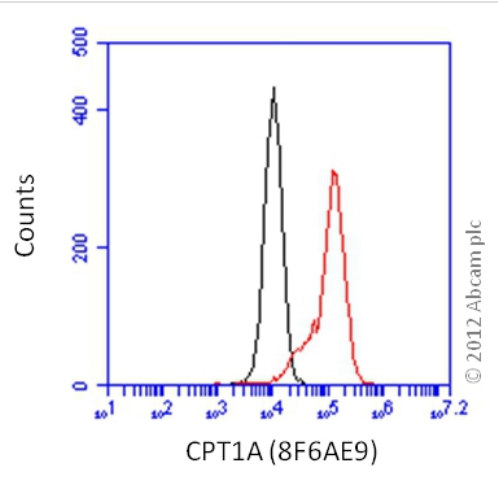
Predicted partial recombinant protein band size : 32 kDa
Observed band size : 32 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CPT1A antibody [8F6AE9] (ab128568)

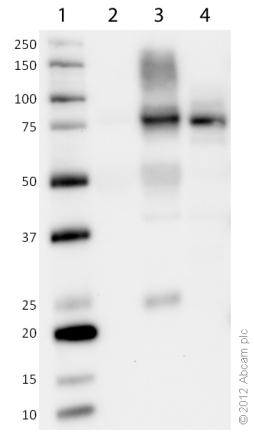
IHC image of CPT1A staining in human normal kidney formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab128568, 0.5µg/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.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Flow Cytometry - Anti-CPT1A antibody [8F6AE9] (ab128568)

Overlay histogram showing HeLa cells stained with ab128568 (red line) or no primary antibody (black line). The cells were fixed with 4% paraformaldehyde (15 min) and then permeabilized with 0.1% Triton X-100 in PBS, 3% BSA for 10 min. The cells were then incubated in 3% BSA in PBS for 10 minutes to block non-specific protein-protein interactions followed by the antibody (ab128568, 1µg/mL) for 60 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96871) at 1/1000 dilution for 30 min.



Western blot - Anti-CPT1A antibody [8F6AE9] (ab128568)

All lanes : Anti-CPT1A antibody [8F6AE9] (ab128568) at 1 µg/ml

Lane 1 : Marker
Lane 2 : HHH (Human heart homogenate) RIPA Extract Immunoprecipitated with no primary antibody
Lane 3 : HHH (Human heart homogenate) RIPA Extract Immunoprecipitated with Anti-CPT1A antibody 8F6AE9 (ab128568)
Lane 4 : HHH (Human heart homogenate) RIPA Extract 20 µg

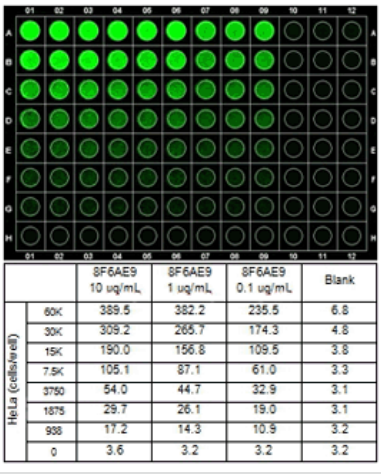
Secondary
All lanes : Goat polyclonal to Mouse IgG – H&L – Pre-Adsorbed (HRP) at 1:10000

Developed using the ECL technique.

Performed under non-reducing conditions.

Predicted band size: 88 kDa

Exposure time: 1 minute



HeLa (cells/well)	8F6AE9 10 ug/mL	8F6AE9 1 ug/mL	8F6AE9 0.1 ug/mL	Blank
60K	389.5	382.2	235.5	6.8
30K	309.2	265.7	174.3	4.8
15K	190.0	156.8	109.5	3.8
7.5K	105.1	87.1	61.0	3.3
3750	54.0	44.7	32.9	3.1
1875	29.7	26.1	19.0	3.1
938	17.2	14.3	10.9	3.2
0	3.6	3.2	3.2	3.2

In-Cell ELISA - Anti-CPT1A antibody [8F6AE9] (ab128568)

In-Cell ELISA for Anti-CPT1A antibody (ab128568) stained HeLa cells (human)
Seeding: HeLa cells seeded in a 1:2 dilution series starting at 60,000 cells/well across Row A, 30,000 cells/well across Row B, etc. Row H contains no cells.
Table: Example In-Cell ELISA Average Data from shown plate.
Fixation: 4% paraformaldehyde PBS fixed for 15 minutes
Permeabilization: 0.3% Triton X-100 PBS for 30 minutes at room temperature while shaking
Blocking: 2x Sigma Block 0.3% Triton X-100 H2O for 2 hours at room temperature while shaking
Primary antibodies: All primaries diluted in 1x Sigma Block with 0.3% Triton X-100 incubated overnight at 4 °C.
• Columns 1-3: Anti-CPT1A antibody (ab128568) 10 ug/mL
• Columns 4-6: Anti-CPT1A antibody (ab128568) 1 ug/mL

• Columns 7-9: Anti-CPT1A antibody (ab128568) 0.1 ug/mL

• Columns 10-12: No Primary

Washing: Briefly 4x with 0.3% TWEEN-20 PBS

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