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Product datasheet

Anti-CPT1A antibody [8F6AE9] ab128568 呕評価済

★★★★★ <u>5 Abreviews</u> <u>148 References</u> 画像数 7

製品の概要

| 製品名 | Anti-CPT1A antibody [8F6AE9] | | | | | | | |
|--------------|---|--|--|--|--|--|--|--|
| 製品の詳細 | Mouse monoclonal [8F6AE9] to CPT1A | | | | | | | |
| 由来種 | Mouse | | | | | | | |
| アプリケーション | 適用あり: Flow Cyt, IHC-P, WB, ICC/IF, In-Cell ELISA | | | | | | | |
| 種交差性 | を差種: Mouse, Rat, Human | | | | | | | |
| 免疫原 | Recombinant fragment, corresponding to amino acids 489-773 of Human CPT1A. | | | | | | | |
| ポジティブ・コントロール | 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 | | | | | | | |
| 特記事項 | 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. | | | | | | | |
| | 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 grips 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 | | | | | | | |
| 製品の特性 | | | | | | | | |
| 製品の状態 | Liquid | | | | | | | |
| 保存方法 | Shipped at 4°C. Store at +4°C. Do Not Freeze. | | | | | | | |

バッファー

Shipped at 4°C. Store at +4°C. Do Not Freeze. pH: 7.4 Preservative: 0.02% Sodium azide Constituent: HEPES buffered saline

| 精製度 | Ammonium Sulphate Precipitation |
|----------|--|
| 特記事項(精製) | 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. |
| ポリ/モノ | モノクローナル |
| クローン名 | 8F6AE9 |
| アイソタイプ | lgG2b |
| 軽鎖の種類 | kappa |

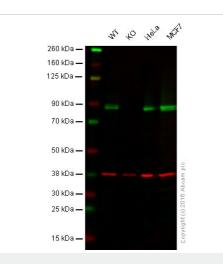
アプリケーション

The Abpromise guaranteeAbpromise保証は、
次のテスト済みアプリケーションにおけるab128568の使用に適用されますアプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

| アプリケーション | Abreviews | 特記事項 |
|---------------|--------------------------|---|
| Flow Cyt | | Use at an assay dependent concentration. |
| IHC-P | ★★★★ <u></u> (<u>1)</u> | Use a concentration of 0.5 μ g/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. |
| WB | ★ ★ ★ ★ ★ <u>(3)</u> | Use a concentration of 1 $\mu\text{g/ml}.$ Predicted molecular weight: 88 kDa. |
| ICC/IF | ★ ★ ★ ★ ★ (1) | Use a concentration of 0.5 - 5 μ g/ml. |
| In-Cell ELISA | | Use a concentration of 0.1 - 1 μ g/ml. |

ターゲット情報

| 組織特異性 パスウェイ | Strong expression in kidney and heart, and lower in liver and skeletal muscle. Lipid metabolism; fatty acid beta-oxidation. |
|----------------|---|
| 関連疾患 | 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. |
| 配列類似性 | Belongs to the carnitine/choline acetyltransferase family. |
| 細胞内局在 | Mitochondrion outer membrane. |



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, <u>ab181602</u>, 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.

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

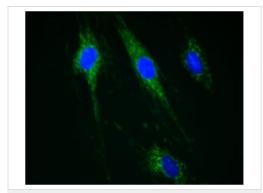
Blocking: 2x Sigma Block 0.1% Triton X-100 H2O for 2 hours at room temperature while rocking

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.

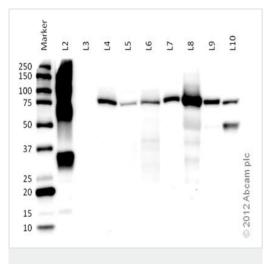
Secondary antibodies: Alexa 488 GAM 1:1000 diluted in 1% NGS with 0.1% Triton X-100 PBS incubated for 2 hours at room temperature while rocking.

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.



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



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 (<u>ab128569</u>) 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 μ g

- 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 lgG – 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

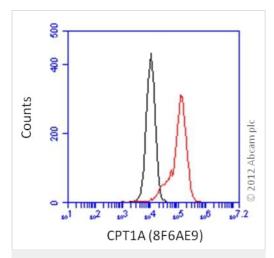
Exposure time: 1 minute

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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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 BondTM system using the standard protocol F. The section was pretreated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab128568, 0.5μ g/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, 1 μ g/mL) for 60 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse lgG (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

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|-----------|--|---------------|--------------------|-------------------|-------------------------|----------------------|------------------|------------|---------------------|---------|-------------------|--------|
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| н | С | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 91 | 62 | 63 | | | 05 07 | | 08 | 08 09 10 | | 11 12 | |
| | | | 8F6AE9 10 ug/mL | | | 8F6AE9 1 ug/mL | | | 8F6AE9 0.1 ug/mL | | Blank | |
| \square | | 60K | 1 | 389.5 | | 382.2 | | 13 | 235.5 | | 6.8 | |
| ~ | | 30K | 1 | 309.2 | | 265.7 | | | 174.3 | | 4.8 | |
| n ell | (III-a v SII- 15K 7.5K 3750 1875 1875 | | | 190.0 105.1 | | 156.8 87.1 | | | 109.5 61.0 | | 3.8 3.3 | |
| Alls/ | | | | | | | | | | | | |
| ě | 1 | 3750 | | 54.0 29.7 | | 44.7 26.1 14.3 | | | 32.9 | | 3.1 3.1 3.2 | |
| ale | | 1875 | | | | | | 19.0 | | | | |
| - | | 938 | 17.2 | | | | | | 10.9 | | | |
| | | 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

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