abcam

Product datasheet

Human CPT1A knockout HEK-293T cell lysate ab256880

4 Images

Overview

Product name	Human CPT1A knockout HEK-293T cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 17 bp deletion in exon 3 and Insertion of the selection cassette in exon 3.
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. **Usage of SDS sample buffer is not recommended with these lyophilized lysates.*
Notes	**Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are prepared. **User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage. Access thousands of knockout cell lysates, generated from commonly used cancer cell lines. **See here for more information on knockout cell lysates.** Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses. This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our **limited use license** and **patent pages**.
Tested applications	**Suitable for:** WB

Properties

Storage instructions Store at -80°C. Please refer to protocols.

Components	1 kit
ab260932 - Human CPT1A knockout HEK293T cell lysate	1 x 100µg
ab255553 - Human wild-type HEK293T cell lysate	1 x 100µg

Cell type epithelial

STR Analysis Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01: 7, 9.3 TPOX: 11 CSF1PO: 11, 12

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.

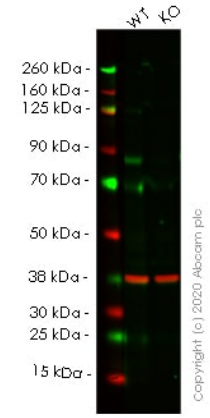
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab256880 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
WB		Use at an assay dependent concentration. Predicted molecular weight: 88 kDa.

Images



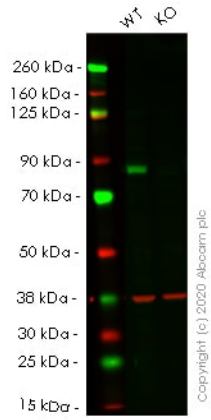
Western blot - Human CPT1A knockout HEK293T cell lysate (ab256880)

Lane 1: Wild-type HEK-293T cell lysate (20µg)

Lane 2: CPT1A knockout HEK-293T cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - **ab234111** observed at 88 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab234111 Anti-CPT1A antibody [EPR21843-71-2F] was shown to specifically 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 and CPT1A knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab234111** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 dilution and 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.



Western blot - Human CPT1A knockout HEK293T cell lysate (ab256880)

Lane 1: Wild-type HEK-293T cell lysate (20µg)

Lane 2: CPT1A knockout HEK-293T cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - **ab220789** observed at 88 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab220789 Anti-CPT1A antibody [EPR21843-71-1C] was shown to specifically 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 and CPT1A knockout samples 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**) were incubated overnight at 4˚C at 1 in 1000 dilution and 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.

Sanger Sequencing - Human CPT1A knockout HEK293T cell lysate (ab256880)

Allele-1: 17 bp deletion in exon 3

Sanger Sequencing - Human CPT1A knockout HEK293T cell lysate (ab256880)

Allele-2: Insertion of the selection cassette in exon 3

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