abcam

Product datasheet

Human CPT2 knockout HeLa cell lysate ab257180

3 Images

Overview

Product name Human CPT2 knockout HeLa cell lysate

Product overview

Knockout cell lysate achieved by CRISPR/Cas9.

Parental Cell Line HeLa

Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon1 and 2 bp deletion in exon1.

Passage number <20

Knockout validation Sanger Sequencing, Western Blot (WB)

Reconstitution notesTo 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 $\ensuremath{\mathsf{REACH}}$

Authorisation, and any other relevant authorisations, for their intended uses.

This product is subject to limited use licenses from The Broad Institute and ERS Genomics

Limited, and is developed with patented technology. For full details of the limited use licenses and

relevant patents please refer to our limited use license and patent pages.

Tested applications Suitable for: WB

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Properties

Storage instructions

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

Components	1 kit
ab260915 - Human CPT2 knockout HeLa cell lysate	1 x 100µg
ab255929 - Human wild-type HeLa cell lysate	1 x 100µg

Cell type epithelial

Disease Adenocarcinoma

Gender Female

STR Analysis Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18

TH01: 7 TPOX: 8,12 CSF1PO: 9, 10

Target

Pathway

Lipid metabolism; fatty acid beta-oxidation.

Involvement in disease

Defects in CPT2 are the cause of carnitine palmitoyltransferase 2 deficiency (CPT2D) [MIM:255110, 600649]; also known as CPT-II deficiency or CPT2 deficiency. CPT2D is an autosomal recessive disorder characterized by recurrent myoglobinuria, episodes of muscle pain, stiffness, and rhabdomyolysis. These symptoms are triggered by prolonged exercise, fasting or viral infection and patients are usually young adults. In addition to this classical, late-onset, muscular type, a hepatic or hepatocardiomuscular form has been reported in infants. Clinical pictures in these children or neonates include hypoketotic hypoglycemia, liver dysfunction, cardiomyopathy and sudden death.

Defects in CPT2 are the cause of carnitine palmitoyltransferase 2 deficiency, lethal neonatal (CPT2D-LN) [MIM:608836]; also known as lethal neonatal CPT-II deficiency. It is a lethal neonatal form of CPT2D. This rarely presentation is antenatal with cerebral periventricular cysts and cystic dysplastic kidneys. The clinical variability of the disease is likely attributed to the variable residual

enzymatic activity.

Sequence similarities

Belongs to the carnitine/choline acetyltransferase family.

Cellular localization

Mitochondrion inner membrane.

Applications

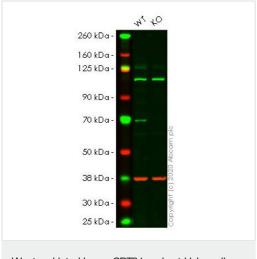
The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab257180 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.

Images



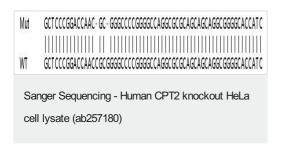
Western blot - Human CPT2 knockout HeLa cell lysate (ab257180)

Lane 1: Wild-type HeLa cell lysate (20µg)

Lane 2: CPT2 knockout HeLa cell lysate (20µg)

Lanes 1-2: Merged signal (red and green). Green - <u>ab181114</u> observed at 74 kDa. Red - loading control <u>ab8245</u> observed at 37 kDa.

ab181114 Anti-CPT2/CPT1 antibody [EPR13626] - C-terminal was shown to specifically react with CPT2/CPT1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab265931 (knockout cell lysate ab257180) was used. Wild-type and CPT2/CPT1 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. ab181114 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 lgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Allele-1: 2 bp deletion in exon1

	Mut	GCTCCCGGACCAAC-GCGGGGCCCCGGGGCCAGGCGCGCAGCAGCAGCAGCGGGGCACCATC			
	WT	GCTCCCGGACCAACCGCGGGCCCCGGGGCCAGGCGCGCAGCAG			
Sanger Sequencing - Human CPT2 knockout HeLa					
cell lysate (ab257180)					

Allele-2: 1 bp deletion in exon1

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