abcam

Product datasheet

Human HADHA knockout HEK-293T cell line ab266274

4 Images

Overview

Product name	Human HADHA knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon 1 and 8 bp deletion in exon 1
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	**Recommended control:** Human wild-type HEK293T cell line (**ab255449**). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** DMEM (High Glucose) + 10% FBS **Initial handling guidelines:** Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability. 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution. 3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of $2x10^4$ cells/cm^2. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C incubator with 5% CO_2. Cultures should be monitored daily. **Subculture guidelines:** All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $2x10^4$ cells/cm^2 is recommended. A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.

Cells should be passaged when they have achieved 80-90% confluence.
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**.

We will provide viable cells that proliferate on revival.

Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Kidney
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
Antibiotic resistance	Puromycin 1.00µg/ml
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

Target

Function	Bifunctional subunit.
Pathway	Lipid metabolism; fatty acid beta-oxidation.
Involvement in disease	Defects in HADHA are a cause of trifunctional protein deficiency (TFP deficiency) [MIM:609015]. The clinical manifestations are very variable and include hypoglycemia, cardiomyopathy and sudden death. Phenotypes with mainly hepatic and neuromyopathic involvement can also be distinguished. Biochemically, TFP deficiency is defined by the loss of all enzyme activities of the TFP complex. Defects in HADHA are the cause of long-chain 3-hydroxyl-CoA dehydrogenase deficiency (LCHAD deficiency) [MIM:609016]. The clinical features are very similar to TFP deficiency. Biochemically, LCHAD deficiency is characterized by reduced long-chain 3-hydroxyl-CoA dehydrogenase activity, while the other enzyme activities of the TFP complex are normal or only slightly reduced. Defects in HADHA are a cause of maternal acute fatty liver of pregnancy (AFLP) [MIM:609016]. AFLP is a severe maternal illness occurring during pregnancies with affected fetuses. This disease is associated with LCHAD deficiency and characterized by sudden unexplained infant death or hypoglycemia and abnormal liver enzymes (Reye-like syndrome).
Sequence similarities	In the N-terminal section; belongs to the enoyl-CoA hydratase/isomerase family. In the central section; belongs to the 3-hydroxyacyl-CoA dehydrogenase family.
Cellular localization	Mitochondrion.

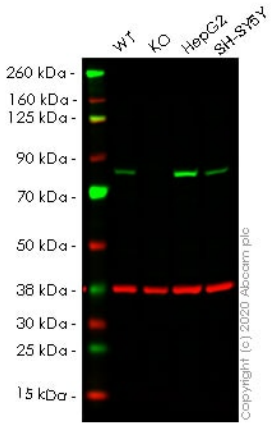
Applications

The Abpromise guarantee	Our **Abpromise guarantee** covers the use of ab266274 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: 83 kDa.

Images



Western blot - Human HADHA knockout HEK293T cell line (ab266274)

All lanes : Anti-HADHA antibody [EPR17940] (**ab203114**) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : HADHA knockout HEK-293T cell lysate
Lane 3 : HepG2 cell lysate
Lane 4 : SH-SY5Y cell lysate

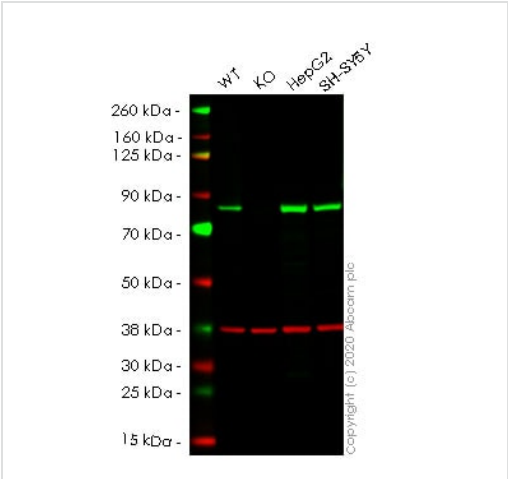
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 83 kDa
Observed band size: 82 kDa

Lanes 1-4: Merged signal (red and green). Green - **ab203114** observed at 82 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab203114 Anti-HADHA antibody [EPR17940] was shown to specifically react with HADHA in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab266274 (knockout cell lysate **ab257464**) was used. Wild-type and HADHA knockout samples were subjected to SDS-PAGE. **ab203114** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 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 HADHA knockout HEK293T cell line (ab266274)

All lanes : Anti-HADHA antibody [EPR17939] (**ab200652**) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : HADHA knockout HEK-293T cell lysate
Lane 3 : HepG2 cell lysate
Lane 4 : SH-SY5Y cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 83 kDa
Observed band size: 82 kDa

Lanes 1-4: Merged signal (red and green). Green - **ab200652** observed at 82 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab200652 Anti-HADHA antibody [EPR17939] was shown to specifically react with HADHA in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab266274 (knockout cell lysate **ab257464**) was used. Wild-type and HADHA knockout samples were subjected to SDS-PAGE. **ab200652** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 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 HADHA knockout HEK293T cell line (ab266274)

Allele-1: 8 bp deletion in exon 1

Sanger Sequencing - Human HADHA knockout HEK293T cell line (ab266274)

Allele-2: 1 bp deletion in exon 1.

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