

Human HADHA knockout HEK-293T cell lysate ab257464

4 图像

概述

产品名称	人HADHA knockout HEK-293T cell裂解物
产品概述	Knockout cell lysate achieved by CRISPR/Cas9.
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)
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.*

说明

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.](#)

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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.

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经测试应用

适用于: WB

性能

存放说明 Store at -80°C. Please refer to protocols.

组件	1 kit
ab260245 - Human HADHA 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

靶标

功能 Bifunctional subunit.

通路 Lipid metabolism; fatty acid beta-oxidation.

疾病相关 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).

序列相似性 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.

细胞定位 Mitochondrion.

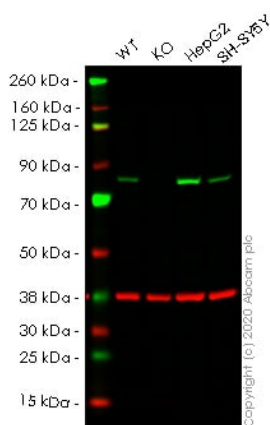
应用

The Abpromise guarantee **Abpromise™**承诺保证使用ab257464于以下的经测试应用

“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB		Use at an assay dependent concentration. Predicted molecular weight: 83 kDa.

图片



Western blot - Human HADHA knockout HEK293T cell lysate (ab257464)

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

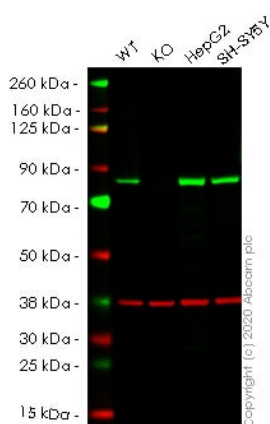
Lane 2: HADHA knockout HEK-293T cell lysate (20 µg)

Lane 3: HepG2 cell lysate (20 µg)

Lane 4: SH-SY5Y cell lysate (20 µg)

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 lysate (ab257464)

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

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

Lane 3: HepG2 cell lysate (20 µg)

Lane 4: SH-SY5Y cell lysate (20 µg)

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.

Mut	TGCCGGGCGATTGGCATCCTCAGCCGCTTT-----CAGGATCCTCCGCTCCCGAGGT
WT	TGCCGGGCGATTGGCATCCTCAGCCGCTTTCTGCCTTCAGGATCCTCCGCTCCCGAGGT
Sanger Sequencing - Human HADHA knockout	
HEK293T cell lysate (ab257464)	

Allele-1: 8 bp deletion in exon 1

Mut	TGCCGGGCGATTGGCATCCTCAGCCGCTTT-CTGCCTTCAGGATCCTCCGCTCCCGAGGT
WT	TGCCGGGCGATTGGCATCCTCAGCCGCTTTCTGCCTTCAGGATCCTCCGCTCCCGAGGT
Sanger Sequencing - Human HADHA knockout	
HEK293T cell lysate (ab257464)	

Allele-2: 1 bp deletion in exon 1

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