abcam

Product datasheet

Human ETFA knockout HEK-293T cell lysate ab257943

2 Images

Overview

Product name	Human ETFA 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, Homozygous: Insertion of the selection cassette 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.	
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). <i>This means that the protein of interest is denatured.</i> If you require a native form of the protein please use the live cell version - found <u>here</u> . 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	

Tested applications

Suitable for: WB

Properties

Storage instructions Store at -80°C. Please refer to protocols.			
Components		1 kit	
ab260398 - Human ETFA 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		

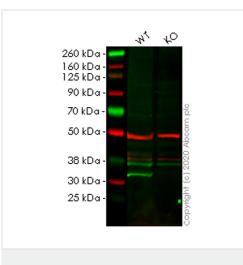
Target	
Function	The electron transfer flavoprotein serves as a specific electron acceptor for several dehydrogenases, including five acyl-CoA dehydrogenases, glutaryl-CoA and sarcosine dehydrogenase. It transfers the electrons to the main mitochondrial respiratory chain via ETF-ubiquinone oxidoreductase (ETF dehydrogenase).
Involvement in disease	Glutaric aciduria 2A
Sequence similarities	Belongs to the ETF alpha-subunit/FixB family.
Domain	Domain I shares an identical polypeptide fold with the beta subunit ETFB though there is no sequence similarity.
Post-translational modifications	The N-terminus is blocked.
Cellular localization	Mitochondrion matrix.

Applications

The Abpromise guaranteeOur Abpromise guaranteecovers the use of ab257943 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: 35 kDa.

Images



Western blot - Human ETFA knockout HEK293T cell Iysate (ab257943) Lane 1: Wild-type HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate (40 ug)

Lane 2: ETFA knockout HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate (40 ug)

ab110316 was shown to specifically react with ETFA in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line **ab266513** (knockout cell lysate ab257943) was used. Wildtype and ETFA knockout samples were subjected to SDS-PAGE. **ab110316** and Anti-beta Tubulin [EP1331Y] - Microtubule Marker (**ab52901**) 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[®] 680RD) preadsorbed (**ab216777**) and Goat anti-Mouse IgG H&L (IRDye[®] 800CW) preadsorbed (**ab216772**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Homozygous: Insertion of the selection cassette in exon 1

Mut	GCCCCGGAGCCGCCGCTCGG*****1 ns ert	i on****** AACATGGTCTCCGCTTCCGC
WT	GCCCCGGAGCCGCCGCTCGG	AACATGGTCTCCGCTTCCGC

Sanger Sequencing - Human ETFA knockout

HEK293T cell lysate (ab257943)

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