

Product datasheet

Human ETFA knockout HEK293T cell line ab266513

3 Images

Overview

Product name	Human ETFA knockout HEK293T cell line
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)
Tested applications	Suitable for: WB
Biosafety level	2
General notes	<p>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.</p> <p>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: DMEM (High Glucose) + 10% FBS</p> <p>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.</p> <ol style="list-style-type: none"> 1. Thaw the vial in 37°C water bath 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 (Click here to view haemocytometer protocol) or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of 2x10⁴ cells/cm². This should allow for confluency within 48 hours. 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% CO₂. Cultures should be monitored daily. <p>Subculture guidelines:</p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of 2x10⁴ cells/cm² is recommended for confluency (80-90% confluence)</p>

within 48 hours.

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.

[Click here to view the Mammalian cell tissue culture protocol](#)

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Properties

Number of cells	1 x 10 ⁶ cells/vial, 1 mL
Viability	~90%
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
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% DMSO, 2% Cellulose, methyl ether
Purity	Immunogen affinity purified

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.

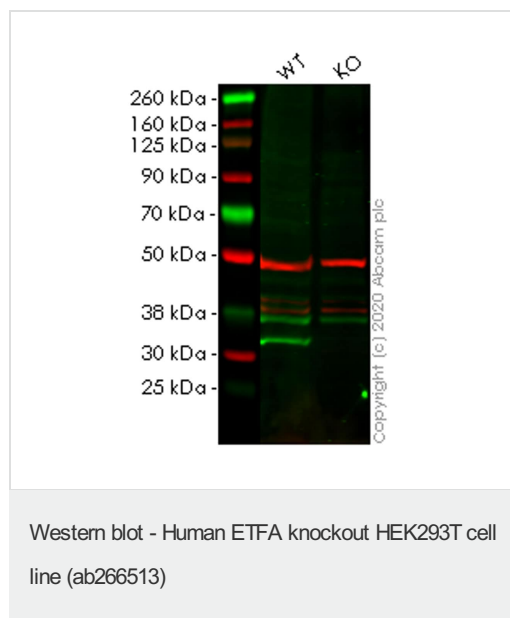
Applications

Our [Abpromise guarantee](#) covers the use of **ab266513** 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



All lanes : Anti-ETFA antibody [2B11AE8] ([ab110316](#)) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

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

Lysates/proteins at 40 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed ([ab216777](#)) at 1/10000 dilution

Predicted band size: 35 kDa

Observed band size: 35 kDa

Lanes 1-2: Merged signal (red and green). Green - [ab110316](#) observed at 35 kDa. Red - loading control [ab52901](#) observed at kDa.

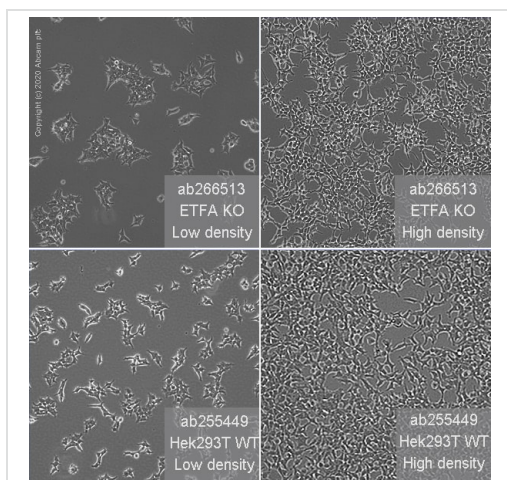
[ab110316](#) Anti-ETFA antibody [2B11AE8] was shown to specifically react with ETFA in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab266513 (knockout cell lysate [ab257943](#)) was used. Wild-type and ETFA knockout samples were subjected to SDS-PAGE. [ab110316](#) and 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.

Mut	GCCCCGGAGCCCGCTCGG*****Insertion*****AACATGGTCTCCGCTTCGGC
WT	GCCCCGGAGCCCGCTCGG AACATGGTCTCCGCTTCGGC

Homozygous: Insertion of the selection cassette in exon 1

Sanger Sequencing - Human ETFA knockout

HEK293T cell line (ab266513)



Human ETFA knockout HEK293T cell line

(ab266513)

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