

Product datasheet

Human FTH1 knockout HEK293T cell line ab266581

2 Images

Overview

Product name Human FTH1 knockout HEK293T cell line

Parental Cell Line HEK293T

Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, Homozygous: 5 bp deletion in exon 1

Passage number <20

Knockout validation Sanger Sequencing, Western Blot (WB)

Tested applications Suitable for: WB

Biosafety level

General notesRecommended 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 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.

Subculture guidelines:

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) 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 10⁶ 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

Antibiotic resistance Puromycin 1.00µg/ml

Mycoplasma free Yes

Storage instructions Shipped on Dry Ice. Store in liquid nitrogen.

Storage buffer Constituents: 8.7% DMSO, 2% Cellulose, methyl ether

Target

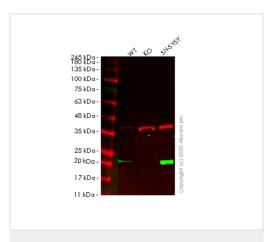
Applications

Our Abpromise guarantee covers the use of ab266581 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: 21 kDa.

Images



Western blot - Human FTH1 knockout HEK293T cell line (ab266581)

All lanes : Anti-Ferritin antibody [EPR3004Y] (ab75973) at 1/1000 dilution

Lane 1: Wild-type HEK293T cell lysate

Lane 2: FTH1 knockout HEK293T cell lysate

Lane 3: SH-SY5Y cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 21 kDa **Observed band size:** 21 kDa

Lanes 1-3: Merged signal (red and green). Green - ab75973 observed at 21 kDa. Red - loading control ab8245 observed at 36 kDa.

ab75973 Anti-Ferritin antibody [EPR3004Y] was shown to specifically react with Ferritin in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266581 (knockout cell lysate ab256924) was used. Wild-type and Ferritin knockout samples were subjected to SDS-PAGE. ab75973 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at room temperature for 2. 5 hours 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.

Homozygous: 5 bp deletion in exon 1

Sanger Sequencing - Human FTH1 knockout

HEK293T cell line (ab266581)

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