abcam

Product datasheet

Human FTL knockout HeLa cell line ab265533

画像数 5

製品の概要

製品名	Human FTL knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon 1 and Insertion of the selection cassette in exon 1
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
アプリケーション	**適用あり:** WB
Biosafety level	2
特記事項	**Recommended control:** Human wild-type HeLa cell line (**ab255928**). 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, 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**.

We will provide viable cells that proliferate on revival.

製品の特性

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Cervix
Cell type	epithelial
Disease	Adenocarcinoma
Gender	Female
STR Analysis	Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18 TH01: 7 TPOX: 8,12 CSF1PO: 9, 10
Mycoplasma free	Yes
保存方法	Shipped on Dry Ice. Store in liquid nitrogen.
バッファー	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

ターゲット情報

機能	Stores iron in a soluble, non-toxic, readily available form. Important for iron homeostasis. Iron is taken up in the ferrous form and deposited as ferric hydroxides after oxidation. Also plays a role in delivery of iron to cells. Mediates iron uptake in capsule cells of the developing kidney.
関連疾患	Defects in FTL are the cause of hereditary hyperferritinemia-cataract syndrome (HHCS) [MIM:600886]. It is an autosomal dominant disease characterized by early-onset bilateral cataract. Affected patients have elevated level of circulating ferritin. HHCS is caused by mutations in the iron responsive element (IRE) of the FTL gene. Defects in FTL are the cause of neurodegeneration with brain iron accumulation type 3 (NBIA3) [MIM:606159]; also known as adult-onset basal ganglia disease. It is a movement disorder with heterogeneous presentations starting in the fourth to sixth decade. It is characterized by a variety of neurological signs including parkinsonism, ataxia, corticospinal signs, mild nonprogressive cognitive deficit and episodic psychosis. It is linked with decreased serum ferritin levels.
配列類似性	Belongs to the ferritin family. Contains 1 ferritin-like diiron domain.

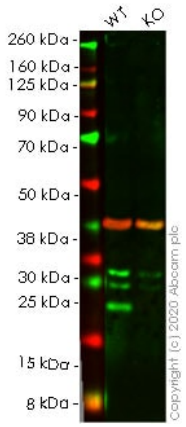
アプリケーション

The Abpromise guarantee	**Abpromise保証は、次のテスト済みアプリケーションにおけるab265533の使用に適用されます**

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Predicted molecular weight: 20 kDa.

画像



Western blot - Human FTL knockout HeLa cell line (ab265533)

All lanes : Anti-Ferritin Light Chain antibody [FTL/1386] (**ab218400**) at 1/500 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : FTL knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

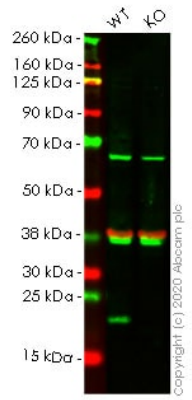
Secondary
All lanes : Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 20 kDa
Observed band size: 20 kDa

Lanes 1-2: Merged signal (red and green). Green - **ab218400** observed at 20 kDa. Red - loading control **ab181602** observed at 37 kDa.

ab218400 Anti-FTL was shown to specifically react with Ferritin in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265533 (knockout cell lysate **ab256926**) was used. Wild-type and FTL knockout samples were subjected to SDS-PAGE. **ab218400** and Anti-GAPDH antibody[EPR16891] - Loading Control (**ab181602**) were incubated overnight at 4°C at 1 in 500 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human FTL knockout HeLa cell line (ab265533)

All lanes : Anti-Ferritin Light Chain antibody [EPR5260] (**ab109373**) at 1/2000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : Ferritin knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

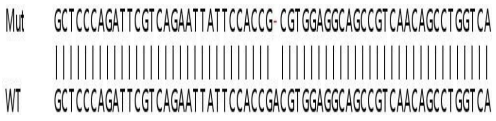
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 20 kDa
Observed band size: 20 kDa

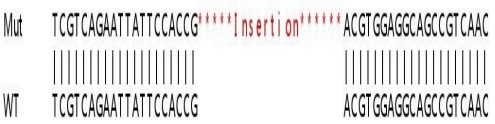
Lanes 1-2: Merged signal (red and green). Green - **ab109373** observed at 20 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab109373 Anti-FTL was shown to specifically react with Ferritin in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265533 (knockout cell lysate **ab256926**) was used. Wild-type and FTL knockout samples were subjected to SDS-PAGE. **ab109373** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 2000 dilution 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.



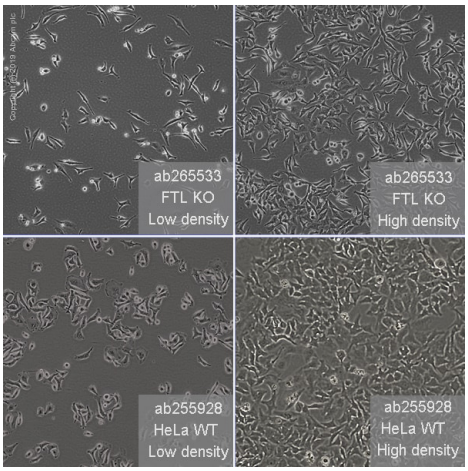
Sanger Sequencing - Human FTL knockout HeLa cell line (ab265533)

Allele-1: 1 bp deletion in exon 1.



Sanger Sequencing - Human FTL knockout HeLa cell line (ab265533)

Allele-2: Insertion of the selection cassette in exon 1.



Cell Culture - Human FTL knockout HeLa cell line (ab265533)

Representative images of FTL knockout HeLa cells, low and high confluency examples (top left and right respectively) and wild-type HeLa cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using a EVOS XL Core microscope.

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