abcam

Product datasheet

Human ASS1 knockout HeLa cell line ab264989

7 图像

概述

产品名称	人ASS1 knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon 3 and Insertion of the selection cassette in exon 3
Passage number	<20
Knockout validation	Immunocytochemistry (ICC), Sanger Sequencing, Western Blot (WB)
经测试应用	**适用于:** WB, ICC
Biosafety level	2
常规说明	**Recommended control:** Human wild-type HeLa cell line (**ab255448**). 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.

性能

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
存放说明	Shipped on Dry Ice. Store in liquid nitrogen.
存储溶液	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

靶标

通路	Amino-acid biosynthesis; L-arginine biosynthesis; L-arginine from L-ornithine and carbamoyl phosphate: step 2/3. Nitrogen metabolism; urea cycle; (N(omega)-L-arginino)succinate from L-aspartate and L-citrulline: step 1/1.
疾病相关	Defects in ASS1 are the cause of citrullinemia type 1 (CTLN1) [MIM:215700]. Citrullinemia belongs to the urea cycle disorders. It is an autosomal recessive disease characterized primarily by elevated serum and urine citrulline levels. Ammonia intoxication is another manifestation. CTLN1 usually manifests in the first few days of life. Affected infants appear normal at birth, but as ammonia builds up in the body they present symptoms such as lethargy, poor feeding, vomiting, seizures and loss of consciousness. Less commonly, a milder CTLN1 form can develop later in childhood or adulthood.
序列相似性	Belongs to the argininosuccinate synthase family. Type 1 subfamily.

应用

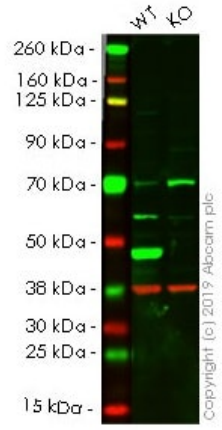
The Abpromise guarantee **Abpromise™承诺保证使用ab264989于以下的经测试应用**

"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

应用	Ab评论	说明
WB		Use at an assay dependent concentration. Predicted molecular weight: 47 kDa.
ICC		Use at an assay dependent concentration.

图片



Western blot - Human ASS1 knockout HeLa cell line (ab264989)

All lanes : Anti-ASS1 antibody [EPR12398] (**ab170952**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ASS1 knockout HeLa cell lysate

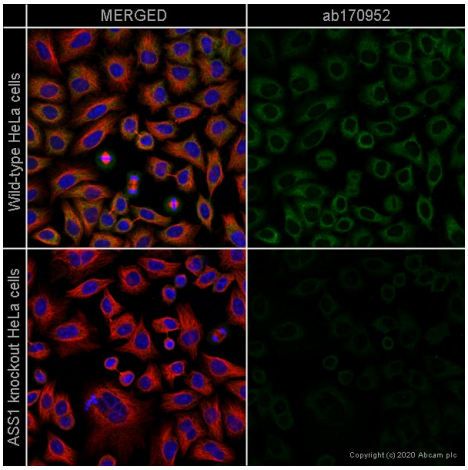
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 47 kDa
Observed band size: 47 kDa

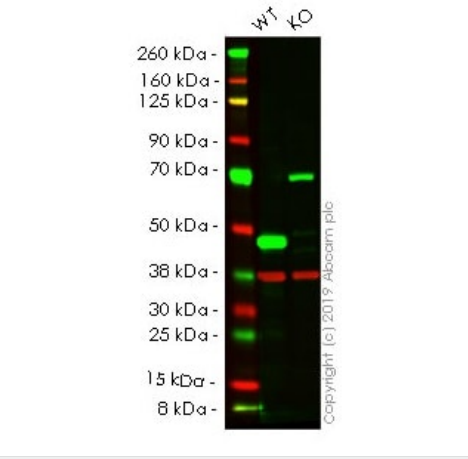
Lanes 1- 2: Merged signal (red and green). Green - **ab170952** observed at 47 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab170952 was shown to react with ASS1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab264989 (knockout cell lysate **ab257143**) was used. Wild-type HeLa and ASS1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab170952** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 dilution and a 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.



Immunocytochemistry/ Immunofluorescence - Human ASS1 knockout HeLa cell line (ab264989)

ab170952 staining ASS1 in wild-type HeLa cells (top panel) and ASS1 knockout HeLa cells (ab264989) (bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab170952** at 1/100 dilution and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Western blot - Human ASS1 knockout HeLa cell line (ab264989)

All lanes : Anti-ASS1 antibody [EPR12399(B)] - C-terminal (**ab170900**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ASS1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

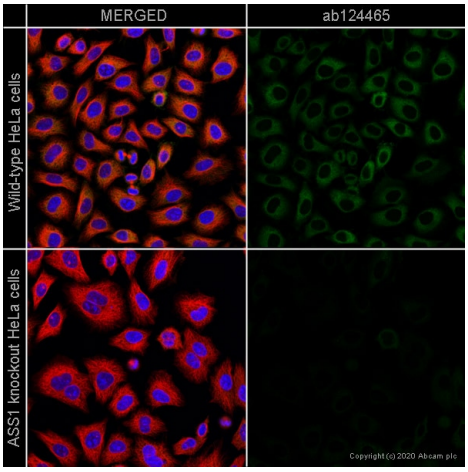
Performed under reducing conditions.

Predicted band size: 47 kDa
Observed band size: 47 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab170900** observed at 47 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab170900 was shown to react with ASS1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab264989 (knockout cell lysate **ab257143**) was used. Wild-type HeLa and ASS1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab170900** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 dilution and a 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.



Immunocytochemistry/ Immunofluorescence - Human ASS1 knockout HeLa cell line (ab264989)

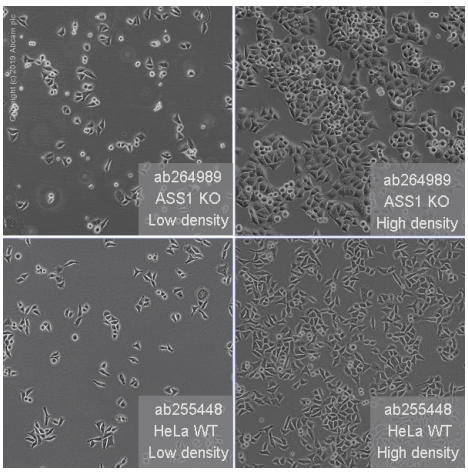
ab124465 staining ASS1 in wild-type HeLa cells (top panel) and ASS1 knockout HeLa cells (ab264989) (bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab124465** at 1/1000 dilution and **ab6046** (Rabbit polyclonal to beta Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to mouse IgG (Alexa Fluor® 488) (**ab150117**) at 2 µg/ml (shown in green) and a goat secondary antibody to rabbit IgG (Alexa Fluor® 594) (**ab150080**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).

Sanger Sequencing - Human ASS1 knockout HeLa cell line (ab264989)

Allele-1: 1 bp insertion in exon 3.

Sanger Sequencing - Human ASS1 knockout HeLa cell line (ab264989)

Allele-2: Insertion of the selection cassette in exon 3.



Cell Culture - Human ASS1 knockout HeLa cell line (ab264989)

Representative images of ASS1 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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