abcam

Product datasheet

Human MTAP knockout HeLa cell line ab265272

6 图像

概述

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

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

功能	Plays a major role in polyamine metabolism and is important for the salvage of both adenine and methionine.
组织特异性	Ubiquitously expressed.
序列相似性	Belongs to the PNP/MTAP phosphorylase family.
细胞定位	Cytoplasm.

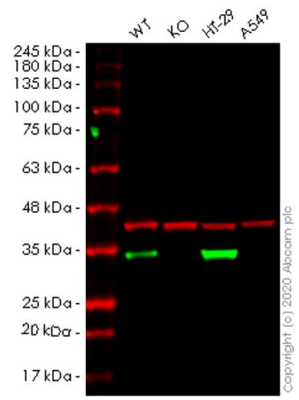
应用

The Abpromise guarantee **Abpromise™承诺保证使用ab265272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
WB		Use at an assay dependent concentration. Predicted molecular weight: 31 kDa.

图片



Western blot - Human MTAP knockout HeLa cell line (ab265272)

All lanes : Anti-MTAP antibody [EPR22570-76] (**ab254265**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : MTAP knockout HeLa cell lysate
Lane 3 : HT-29 cell lysate
Lane 4 : A549 cell lysate

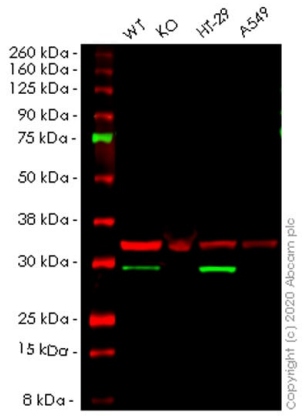
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 31 kDa
Observed band size: 32 kDa

Lanes 1-4: Merged signal (red and green). Green - **ab254265** observed at 32 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab254265 Anti-MTAP antibody [EPR22570-76] was shown to specifically react with MTAP in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265272 (knockout cell lysate **ab257194**) was used. Wild-type and MTAP knockout samples were subjected to SDS-PAGE. **ab254265** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) 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® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.



Western blot - Human MTAP knockout HeLa cell line (ab265272)

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Lane 1 : Wild-type HeLa cell lysate
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Lane 3 : HT-29 cell lysate
Lane 4 : A549 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 31 kDa
Observed band size: 32 kDa

anes 1- 4: Merged signal (red and green). Green - **ab126770** observed at 32 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab126770 Anti-MTAP antibody [EPR6893] was shown to specifically react with MTAP in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265272 (knockout cell lysate **ab257194**) was used. Wild-type and MTAP knockout samples were subjected to SDS-PAGE. **ab126770** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) 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® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.

Sanger Sequencing - Human MTAP knockout HeLa cell line (ab265272)

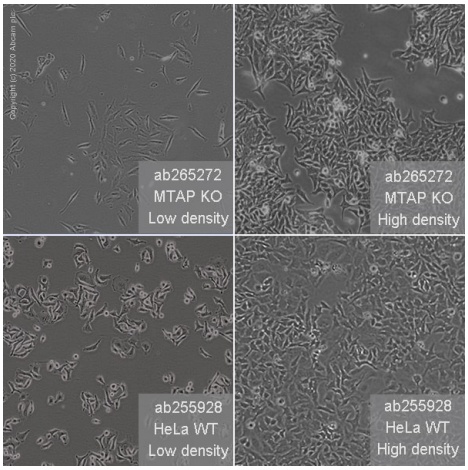
Allele-1: 1 bp insertion in exon 1.

Sanger Sequencing - Human MTAP knockout HeLa cell line (ab265272)

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

Sanger Sequencing - Human MTAP knockout HeLa cell line (ab265272)

Allele-3: Insertion of the selection cassette in exon 1.



Cell Culture - Human MTAP knockout HeLa cell line (ab265272)

Representative images of MTAP 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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