abcam

Product datasheet

Human ABCC1 (MRP1) knockout HeLa cell line ab265256

6 图像

概述

产品名称	人ABCC1 (MRP1) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 14 bp deletion in exon 9 and 2 bp deletion in exon 9
Passage number	<20
Knockout validation	Immunocytochemistry (ICC), Sanger Sequencing, Western Blot (WB)
经测试应用	**适用于:** WB, Flow Cyt, ICC
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 and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant 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
Antibiotic resistance	Puromycin 1.00µg/ml
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	Mediates export of organic anions and drugs from the cytoplasm. Mediates ATP-dependent transport of glutathione and glutathione conjugates, leukotriene C4, estradiol-17-beta-o-glucuronide, methotrexate, antiviral drugs and other xenobiotics. Confers resistance to anticancer drugs. Hydrolyzes ATP with low efficiency.
组织特异性	Lung, testis and peripheral blood mononuclear cells.
序列相似性	Belongs to the ABC transporter superfamily. ABCC family. Conjugate transporter (TC 3.A.1.208) subfamily. Contains 2 ABC transmembrane type-1 domains. Contains 2 ABC transporter domains.
细胞定位	Cell membrane.

应用

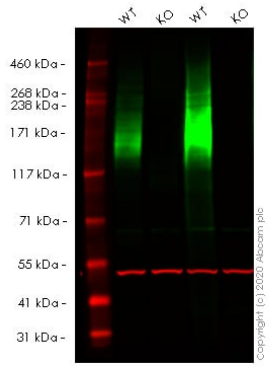
The Abpromise guarantee **Abpromise™承诺保证使用ab265256于以下的经测试应用**

"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: 171 kDa.

应用	Ab评论	说明
Flow Cyt		Use at an assay dependent concentration.
ICC		Use at an assay dependent concentration.

图片



Western blot - Human ABCC1 (MRP1) knockout HeLa cell line (ab265256)

All lanes : Anti-MRP1 antibody [EPR21062] (**ab233383**) at 1/1000 dilution

Lane 1 : Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : MRP1 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 3 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate
Lane 4 : MRP1 knockout A549 (Human lung carcinoma cell line) whole 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

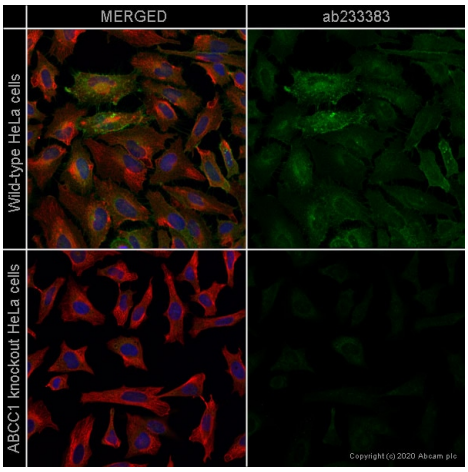
Performed under reducing conditions.

Predicted band size: 171 kDa
Observed band size: 250 kDa

Lanes 1-4: Merged signal (red and green). Green - **ab233383** observed at 250 kDa. Red - loading control **ab7291** observed at 50 kDa.

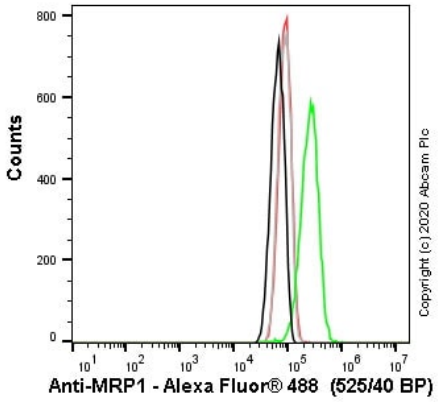
ab233383 Anti-MRP1 antibody [EPR21062] was shown to specifically react with MRP1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265256 (knockout cell lysate **ab257242**) was used. Wild-type and MRP1 knockout samples were subjected to SDS-PAGE. **ab233383** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) 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 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Human ABCC1 (MRP1) knockout HeLa cell line (ab265256)

ab233383 staining MRP1 in wild-type HeLa cells (top panel) and ABCC1 knockout HeLa cells (ab265256) (bottom panel). The cells were fixed with 4% paraformaldehyde (10 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 **ab233383** 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).



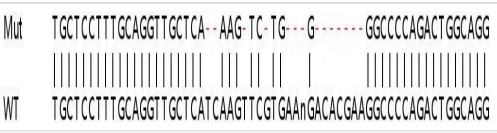
Flow Cytometry (Intracellular) - Human ABCC1 (MRP1) knockout HeLa cell line (ab265256)

Flow cytometry overlay histogram showing wild-type HeLa (green line) and ABCC1 knockout HeLa cells (ab265256) stained with **ab260038** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (**ab260038**) ($1x10^6$ in 100µl at 0.2 µg/ml) for 30 min at 22°C.

The secondary antibody Goat anti-rabbit IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150081**) was used at 1/2000 for 30 min at 22°C.

Isotype control antibody was Rabbit IgG (monoclonal) (**ab172730**) used at the same concentration and conditions as the primary antibody (wild-type HeLa - black line ABCC1 knockout HeLa - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

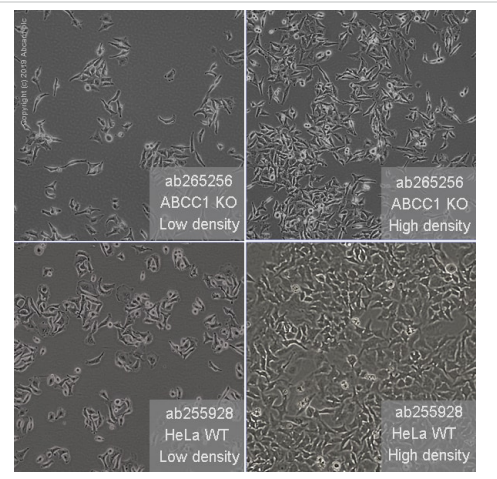


Sanger Sequencing - Human ABCC1 knockout HeLa cell line (ab265256)

Allele-1: 14 bp deletion in exon 9.

Sanger Sequencing - Human ABCC1 knockout HeLa cell line (ab265256)

Allele-2: 2 bp deletion in exon 9.



Cell Culture - Human ABCC1 (MRP1) knockout HeLa cell line (ab265256)

Representative images of ABCC1 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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