abcam

Product datasheet

Human GPX4 knockout HeLa cell line ab262509

1 References 4 图像

概述

产品名称	人GPX4 knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 26 bp deletion, 2 bp insertion; Frameshift: 93.31%
Passage number	<20
Knockout validation	Next Generation Sequencing (NGS), 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 (**ab271142**). 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
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	Protects cells against membrane lipid peroxidation and cell death. Required for normal sperm development and male fertility. Could play a major role in protecting mammals from the toxicity of ingested lipid hydroperoxides. Essential for embryonic development. Protects from radiation and oxidative damage.
组织特异性	Present primarily in testis.
序列相似性	Belongs to the glutathione peroxidase family.
细胞定位	Mitochondrion. Cytoplasm.

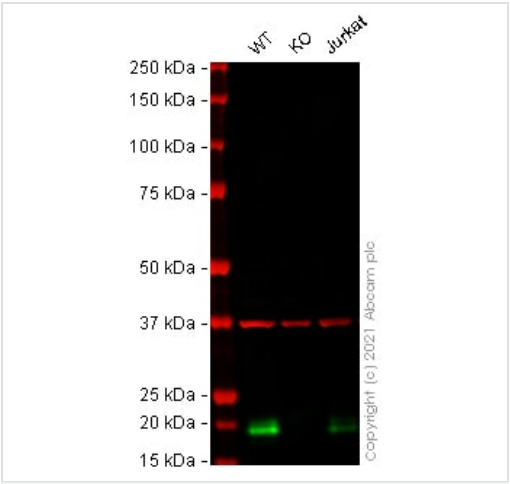
应用

The Abpromise guarantee **Abpromise™承诺保证使用ab262509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: 22 kDa.

图片



Western blot - Human GPX4 knockout HeLa cell line (ab262509)

All lanes : Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] (**ab125066**) at 1/1000 dilution

Lane 1 : Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : GPX4 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 3 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

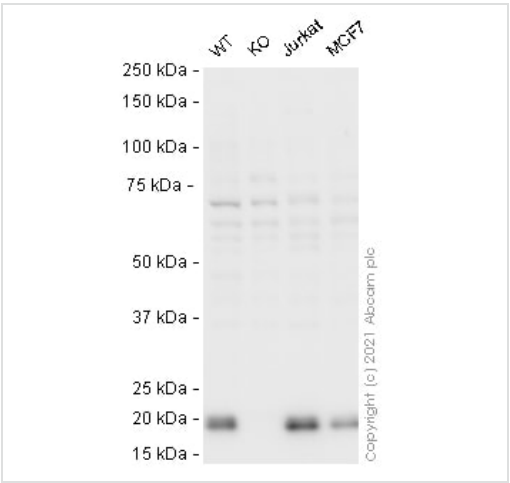
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 22 kDa
Observed band size: 20 kDa

Lanes 1 - 3: Merged signal (red and green). Green - **ab125066** observed at 20 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab125066 was shown to react with Glutathione Peroxidase 4 in wild-type HeLa cells in Western blot with loss of signal observed in GPX4 knockout cell line ab262509 (knockout cell lysate **ab263935**). Wild-type HeLa and GPX4 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab125066** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Human GPX4 knockout HeLa cell line (ab262509)

All lanes : HRP Anti-Glutathione Peroxidase 4 antibody [EPNCIR144] (**ab206266**) at 1/5000 dilution

Lane 1 : Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : GPX4 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 3 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate
Lane 4 : Hep G2 (Human liver hepatocellular carcinoma cell line) whole cell lysate

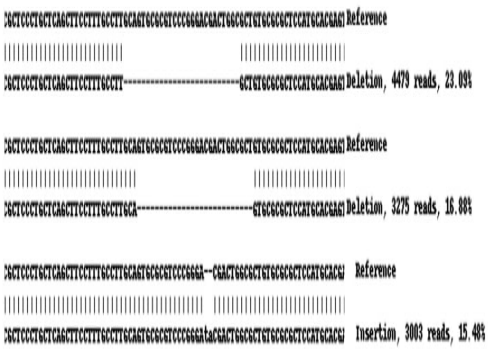
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 22 kDa
Observed band size: 20 kDa

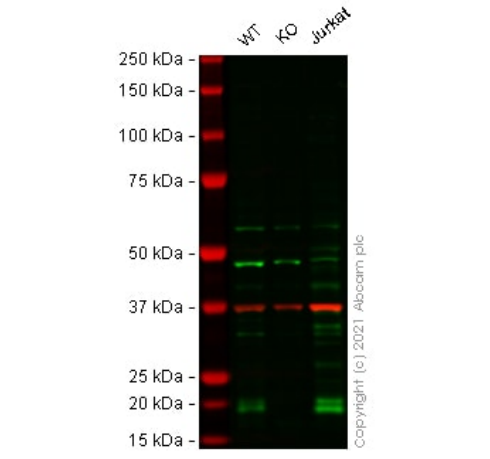
Exposure time: 4 minutes

ab206266 was shown to react with Glutathione Peroxidase 4 (HRP) in wild-type HeLa cells in western blot. Loss of signal was observed when GPX4 knockout cell line ab262509 (knockout cell lysate **ab263935**) was used. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab206266** overnight at 4 °C at a 1 in 5000 dilution Blots were developed with Optiblot ECL reagent (**ab133456**) and imaged.



Next Generation Sequencing - Human GPX4 knockout HeLa cell line (ab262509)

Knockout achieved by CRISPR/Cas9; X = 26 bp deletion, 2 bp insertion; Frameshift: 93.31%



Western blot - Human GPX4 knockout HeLa cell line (ab262509)

All lanes : Anti-Glutathione Peroxidase 4 antibody (**ab41787**) at 1 µg/ml

Lane 1 : Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : GPX4 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 3 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 22 kDa
Observed band size: 20 kDa

Lanes 1 - 3: Merged signal (red and green). Green - **ab41787** observed at 20 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab41787 was shown to react with Glutathione Peroxidase 4 in wild-

type HeLa cells in Western blot with loss of signal observed in GPX4 knockout cell line ab262509 (knockout cell lysate **ab263935**). Wild-type HeLa and GPX4 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab41787** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at 1 µg/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.cn/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors