# abcam

## Product datasheet

# Human GPX4 knockout HeLa cell lysate ab263935

### 画像数 4

#### 製品の概要

製品名 Human GPX4 knockout HeLa cell lysate

製品の概要 Knockout cell lysate achieved by CRISPR/Cas9.

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)

**Reconstitution notes**To use as WB control, resuspend the lyophilizate in 50 μL of LDS\* Sample Buffer to have a final

concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M

DTT.

\*Usage of SDS sample buffer is not recommended with these lyophilized lysates.

特記事項

**Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are prepared.

**User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage.

Access thousands of knockout cell lysates, generated from commonly used cancer cell lines. **See here for more information on knockout cell lysates.** 

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アプリケーション **適用あり**: WB

#### 製品の特性

#### 保存方法

Store at -80°C. Please refer to protocols.

内容	1 kit
ab280488 - Human GPX4 knockout HeLa cell lysate	1 x 100µg
ab269597 - Human wild-type HeLa cell lysate	1 x 100μg

**Cell type** epithelial

**Disease** Adenocarcinoma

**Gender** Female

#### ターゲット情報

機能 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.

組織特異性 Present primarily in testis.

**配列類似性** Belongs to the glutathione peroxidase family.

細胞内局在 Mitochondrion. Cytoplasm.

# アプリケーション

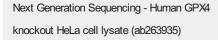
**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab263935の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

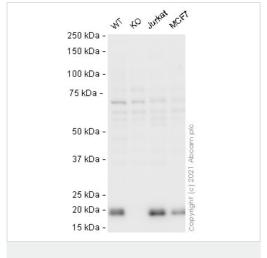
アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Predicted molecular weight: 22 kDa.

#### 画像



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





Western blot - Human GPX4 knockout HeLa cell lysate

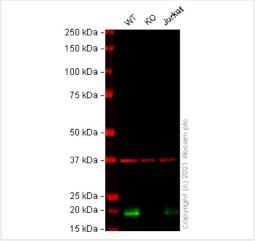
**Lanes 1:** Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate, 20 ug

**Lanes 2:** GPX4 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate, 20 ug

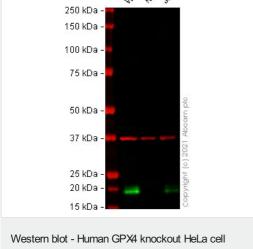
**Lanes 3:** Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate, 20 ug

**Lanes 3:** Hep G2 (Human liver hepatocellular carcinoma cell line) whole cell lysate, 20 ug

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.



lysate



Mr 40 jumper 250 kDa 150 kDa 100 kDa 75 kDa 50 kDa 37 kDa 25 kDa 20 kDa

Western blot - Human GPX4 knockout HeLa cell lysate

Lanes 1: Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate, 20 ug

Lanes 2: GPX4 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate, 20 ug

Lanes 3: Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate, 20 ug

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 lgG 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.

Lanes 1: Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate, 20 ug

Lanes 2: GPX4 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate, 20 ug

Lanes 3: Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate, 20 ug

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 wildtype 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<sup>®</sup> 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.

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