abcam

Product datasheet

Human GPX1 (Glutathione Peroxidase 1) knockout HEK-293T cell line ab266650

3 Images

Overview

Product name Human GPX1 (Glutathione Peroxidase 1) knockout HEK-293T cell line

Parental Cell Line HEK293T
Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 1

Passage number <20

Knockout validation Sanger Sequencing

Tested applications Suitable for: WB, Sanger Sequencing

Biosafety level

General notesRecommended control: Human wild-type HEK293T cell line (<u>ab255449</u>). 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.

 $\textbf{Cryopreservation cell medium:} \ \ \textbf{Cell Freezing Medium-DMSO Serum free media, contains}$

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 2x10⁴ cells/cm². 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% CO₂. 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/cm² is recommended.

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A partial media change 24 hours prior to subculture may be helpful to encourage growth, if

Cells should be passaged when they have achieved 80-90% confluence.

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We will provide viable cells that proliferate on revival.

Properties

Number of cells 1 x 10⁶ cells/vial, 1 mL

Adherent /Suspension Adherent
Tissue Kidney
Cell type epithelial

STR Analysis Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01:

7, 9.3 TPOX: 11 CSF1PO: 11, 12

Antibiotic resistance Puromycin 1.00µg/ml

Mycoplasma free Yes

Storage instructions Shipped on Dry Ice. Store in liquid nitrogen.

Storage buffer Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

Target

Function Protects the hemoglobin in erythrocytes from oxidative breakdown.

Sequence similaritiesBelongs to the glutathione peroxidase family.

Cellular localization Cytoplasm.

Applications

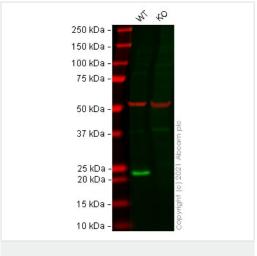
The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab266650 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 22 kDa.
Sanger Sequencing		Use at an assay dependent concentration.

Images



Western blot - Human GPX1 (Glutathione Peroxidase 1) knockout HEK-293T cell line (ab266650)

All lanes : Anti-Glutathione Peroxidase 1 antibody [EPR3311] (ab108429) at 1/1000 dilution

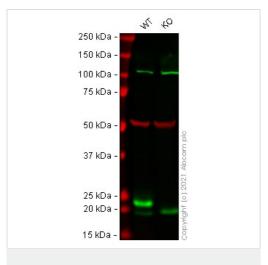
Lane 1: Wild-type HEK-293T cell lysate

Lane 2: GPX1 knockout HEK-293T cell lysate

Performed under reducing conditions.

Predicted band size: 22 kDa **Observed band size:** 22 kDa

False colour image of Western blot: Anti-Glutathione Peroxidase 1 antibody [EPR3311] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab108429 was shown to bind specifically to Glutathione Peroxidase 1. A band was observed at 22 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in GPX1 knockout cell line ab266650 (knockout cell lysate ab256932). To generate this image, wild-type and GPX1 knockout HEK-293T cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.



Western blot - Human GPX1 (Glutathione Peroxidase 1) knockout HEK-293T cell line (ab266650)

All lanes : Anti-Glutathione Peroxidase 1 antibody [EPR3312] (ab108427) at 1/1000 dilution

Lane 1: Wild-type HEK-293T cell lysate

Lane 2: GPX1 knockout HEK-293T cell lysate

Performed under reducing conditions.

Predicted band size: 22 kDa **Observed band size:** 22 kDa

False colour image of Western blot: Anti-Glutathione Peroxidase 1 antibody [EPR3312] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab108427 was shown to bind specifically to Glutathione Peroxidase 1. A band was observed at 22 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in GPX1 knockout cell line ab266650 (knockout cell lysate ab256932). To generate this image, wild-type and GPX1 knockout HEK-293T cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween[®] 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.

Homozygous: 1 bp insertion in exon 1

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