abcam

Product datasheet

Human CAT (Catalase) knockout HeLa cell line ab265250

6 Images

Overview

Product name	Human CAT (Catalase) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon 1 and 4 bp deletion in exon 1 and Insertion of the selection cassette in exon 1
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	**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.
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We will provide viable cells that proliferate on revival.

Properties

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
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

Target

Function	Occurs in almost all aerobically respiring organisms and serves to protect cells from the toxic effects of hydrogen peroxide. Promotes growth of cells including T-cells, B-cells, myeloid leukemia cells, melanoma cells, mastocytoma cells and normal and transformed fibroblast cells.
Involvement in disease	Defects in CAT are the cause of acatalasia (ACATLAS) [MIM:115500]; also known as acatalasemia. This disease is characterized by absence of catalase activity in red cells and is often associated with ulcerating oral lesions.
Sequence similarities	Belongs to the catalase family.
Post-translational modifications	The N-terminus is blocked.
Cellular localization	Peroxisome.

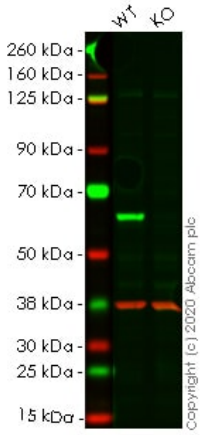
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab265250 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: 60 kDa.

Images



Western blot - Human CAT knockout HeLa cell line (ab265250)

All lanes : Anti-Catalase antibody [EPR20198] - Peroxisome Marker (**ab209211**) at 1/2000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : CAT knockout HeLa cell lysate

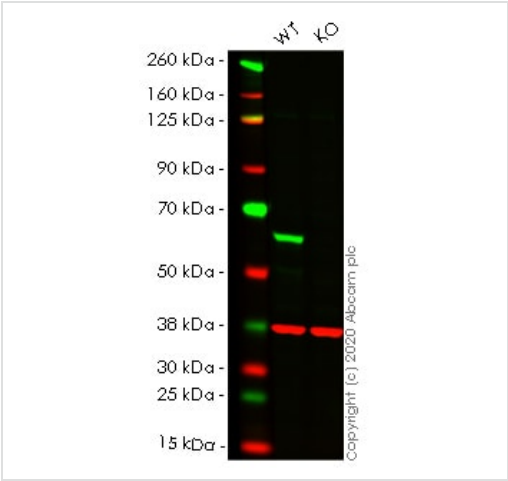
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 60 kDa
Observed band size: 60 kDa

Lanes 1-2: Merged signal (red and green). Green - **ab209211** observed at 60 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab209211 Anti-Catalase antibody [EPR20198] was shown to specifically react with Catalase in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265250 (knockout cell lysate **ab256859**) was used. Wild-type and Catalase knockout samples were subjected to SDS-PAGE. **ab209211** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 2000 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.



Western blot - Human CAT knockout HeLa cell line (ab265250)

All lanes : Anti-Catalase antibody [EP1929Y] - Peroxisome Marker (**ab76024**) at 1/10000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : CAT knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 60 kDa
Observed band size: 60 kDa

Lanes 1-2: Merged signal (red and green). Green - **ab76024** observed at 60 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab76024 Anti-Catalase antibody [EP1929Y] - Peroxisome Marker was shown to specifically react with Catalase in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265250 (knockout cell lysate **ab256859**) was used. Wild-type and Catalase knockout samples were subjected to SDS-PAGE. **ab76024** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 10000 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.

Sanger Sequencing - Human CAT knockout HeLa cell line (ab265250)

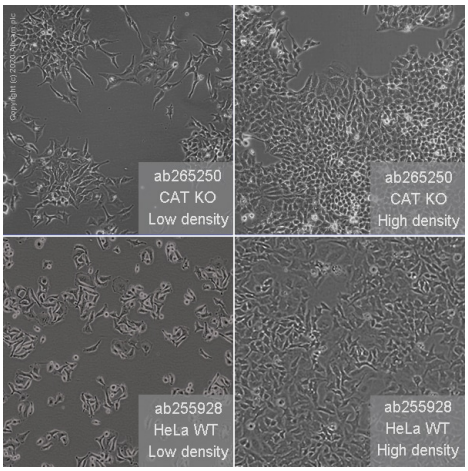
Allele-1: 4 bp deletion in exon 1.

Sanger Sequencing - Human CAT knockout HeLa cell line (ab265250)

Allele-2: 1 bp deletion in exon 1.

Sanger Sequencing - Human CAT knockout HeLa cell line (ab265250)

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



Cell Culture - Human CAT (Catalase) knockout HeLa cell line (ab265250)

Representative images of CAT 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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