abcam

Product datasheet

Human S100A4 knockout HeLa cell line ab265709

7 Images

Overview

Product name	Human S100A4 knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon 2 and 5 bp deletion in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB, Sanger Sequencing
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
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

Tissue specificity	Ubiquitously expressed.
Sequence similarities	Belongs to the S-100 family. Contains 2 EF-hand domains.

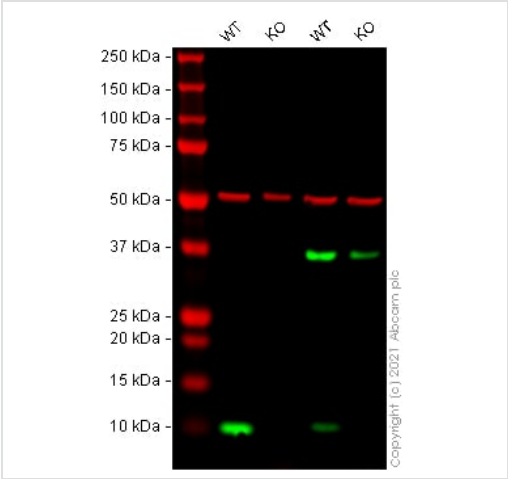
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab265709 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: 12 kDa.
Sanger Sequencing		Use at an assay dependent concentration.

Images



Western blot - Human S100A4 knockout HeLa cell line (ab265709)

All lanes : Anti-S100A4 antibody [EPR14639(2)] (**ab197896**) at 1/1000 dilution

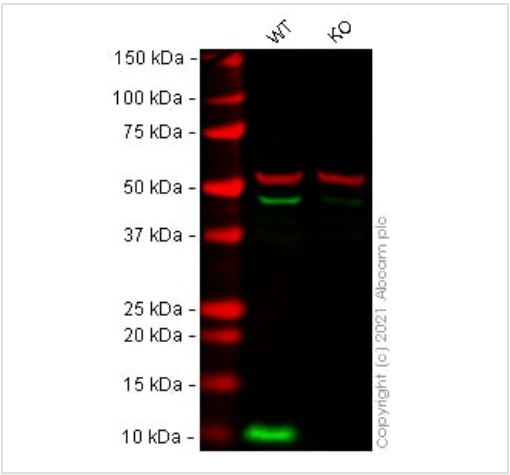
Lane 1 : Wild-type HeLa cell lysate
Lane 2 : S100A4 knockout HeLa cell lysate
Lane 3 : Wild-type A549 cell lysate
Lane 4 : S100A4 knockout A549 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 12 kDa
Observed band size: 11 kDa

False colour image of Western blot: Anti-S100A4 antibody [EPR14639(2)] staining at 1/1000 dilution, shown in green; loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) staining at 1/20000 dilution, shown in red. In Western blot, **ab197896** was shown to bind specifically to S100A4. A band was observed at 11 kDa in wild-type HeLa and A549 cell lysates with no signal observed at this size in S100A4 knockout HeLa cell line ab265709 (knockout cell lysate **ab257046**) and S100A4 knockout A549 cell line **ab261865** (knockout cell lysate **ab261674**). To generate this image, wild-type and S100A4 knockout HeLa and S100A4 knockout A549 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.



Western blot - Human S100A4 knockout HeLa cell line (ab265709)

All lanes : Anti-S100A4 antibody [EPR2761(2)] (**ab124805**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : S100A4 knockout HeLa cell lysate

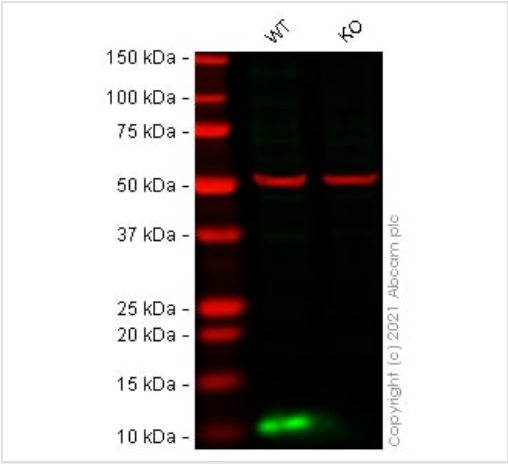
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 12 kDa
Observed band size: 11 kDa

Lanes 1 - 2: Merged signal (red and green). Green - **ab124805** observed at 11 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab124805 was shown to react with S100A4 in wild-type HeLa cells in Western blot with loss of signal observed in S100A4 knockout cell line ab265709 (S100A4 knockout cell lysate **ab257046**). Wild-type HeLa and S100A4 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab124805** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) 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 S100A4 knockout HeLa cell line (ab265709)

All lanes : Anti-S100A4 antibody [EPR14639(2)] (**ab197896**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : S100A4 knockout HeLa cell lysate

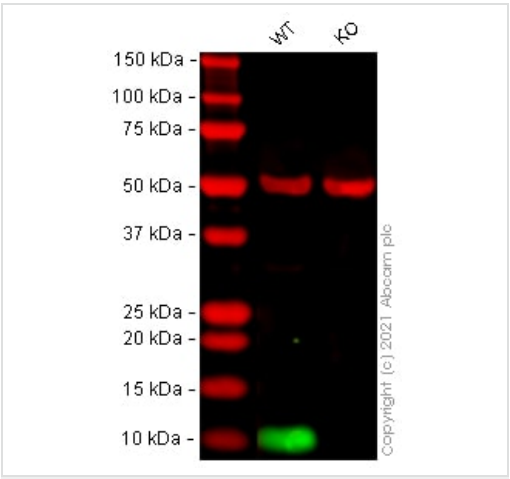
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 12 kDa
Observed band size: 11 kDa

Lanes 1 - 2: Merged signal (red and green). Green - **ab197896** observed at 11 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab197896 was shown to react with S100A4 in wild-type HeLa cells in Western blot with loss of signal observed in S100A4 knockout cell line ab265709 (S100A4 knockout cell lysate **ab257046**). Wild-type HeLa and S100A4 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab197896** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) 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 S100A4 knockout HeLa cell line (ab265709)

All lanes : Anti-S100A4 antibody [S100A4/1481] (**ab218511**) at 0.5 µg/ml

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : S100A4 knockout HeLa cell lysate

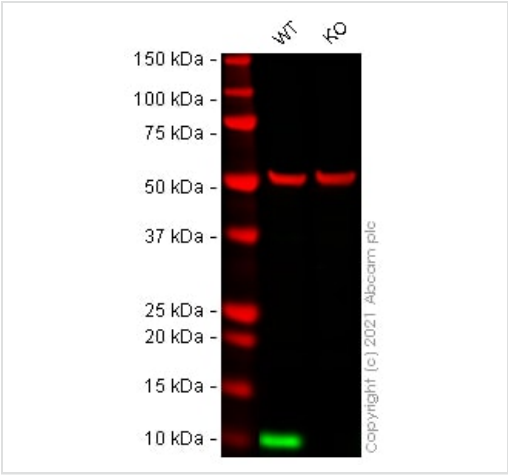
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 12 kDa
Observed band size: 11 kDa

Lanes 1 - 2: Merged signal (red and green). Green - **ab218511** observed at 11 kDa. Red - loading control **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55 kDa.

ab218511 was shown to react with S100A4 in wild-type HeLa cells in Western blot with loss of signal observed in S100A4 knockout cell line ab265709 (S100A4 knockout cell lysate **ab257046**). Wild-type HeLa and S100A4 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab218511** and **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) overnight at 4 °C at 0.5 µg/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Human S100A4 knockout HeLa cell line (ab265709)

All lanes : Anti-S100A4 antibody [S100A4/1482] (**ab218512**) at 0.5 µg/ml

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : S100A4 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 12 kDa
Observed band size: 11 kDa

Lanes 1 - 2: Merged signal (red and green). Green - **ab218512** observed at 11 kDa. Red - loading control **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55 kDa.

ab218512 was shown to react with S100A4 in wild-type HeLa cells in Western blot with loss of signal observed in S100A4 knockout cell line ab265709 (S100A4 knockout cell lysate **ab257046**). Wild-type HeLa and S100A4 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab218512** and **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) overnight at 4 °C at 0.5 µg/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.

Sanger Sequencing - Human S100A4 knockout HeLa cell line (ab265709)

Allele-1: 5 bp deletion in exon 2.

Sanger Sequencing - Human S100A4 knockout HeLa cell line (ab265709)

Allele-2: 1 bp insertion in exon 2.

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