abcam

Product datasheet

Human S100A4 knockout A549 cell lysate ab261674

5 Images

Overview

Product name	Human S100A4 knockout A549 cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	A549
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 1 bp insertion; Frameshift = 95%
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.*
Notes	**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.** Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses. 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**.
Tested applications	**Suitable for:** WB

Properties

Properties

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

Components	1 kit
ab280424 - Human S100A4 knockout A549 cell lysate	1 x 100µg
ab259782 - Human wild-type A549 cell lysate	1 x 100µg

Cell type epithelial

Disease Carcinoma

Gender Male

Target

Tissue specificity Ubiquitously expressed.

Sequence similarities Belongs to the S-100 family.
Contains 2 EF-hand domains.

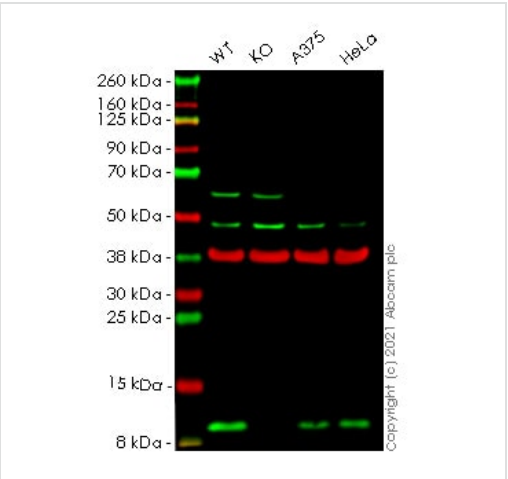
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab261674 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.

Images



Western blot - Human S100A4 knockout A549 cell lysate (ab261674)

Lane 1: Wild-type A549 (Human lung carcinoma cell line) whole cell lysate 20 µg

Lane 2: S100A4 knockout A549 (Human lung carcinoma cell line) whole cell lysate 20 µg

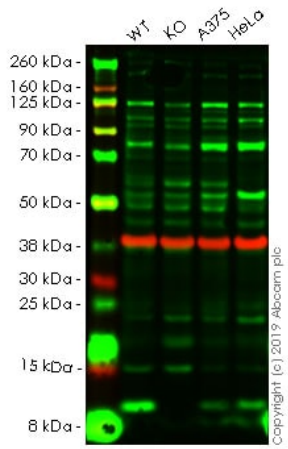
Lane 3: A-375 (Human malignant melanoma cell line) whole cell lysate 20 µg

Lane 4: HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate 20 µg

Lanes 1 - 4: Merged signal (red and green). Green - **ab124805** observed at 12 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab124805 was shown to react with S100A4 in wild-type A549 cells in Western blot with loss of signal observed in S100A4 knockout cell line **ab261865** (knockout cell lysate ab261674). Wild-type A549

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 **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 S100A4 knockout A549 cell lysate (ab261674)

Lane 1: Wild-type A549 (Human lung carcinoma cell line) whole cell lysate 20 ug

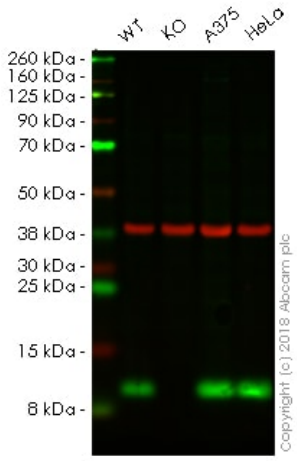
Lane 2: S100A4 knockout A549 (Human lung carcinoma cell line) whole cell lysate 20 ug

Lane 3: A-375 (Human malignant melanoma cell line) whole cell lysate 20 ug

Lane 4: HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate 20 ug

Lanes 1 - 4: Merged signal (red and green). Green - **ab41532** observed at 12 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab41532 was shown to recognize S100A4 in wild-type A549 cells as signal was lost at the expected MW in S100A4 knockout cell line **ab261865** (knockout cell lysate ab261674). Additional cross-reactive bands were observed in the wild-type and knockout samples. Wild-type and S100A4 knockout samples were subjected to SDS-PAGE. Ab41532 and **ab8245** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/250 dilution and 1/20000 dilution respectively. Blots were developed 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 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human S100A4 knockout A549 cell lysate (ab261674)

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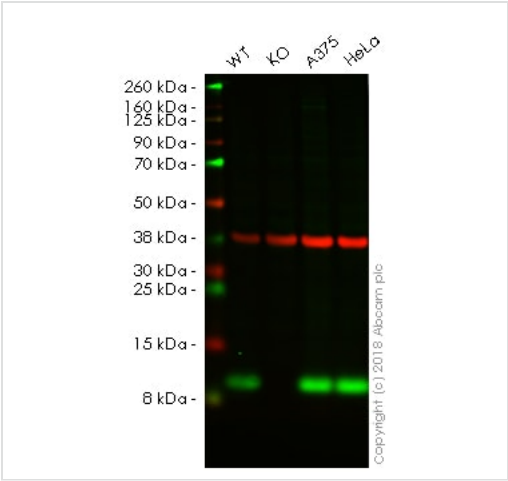
Lane 2: S100A4 knockout A549 (Human lung carcinoma cell line) whole cell lysate 20 ug

Lane 3: A-375 (Human malignant melanoma cell line) whole cell lysate 20 ug

Lane 4: HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate 20 ug

Lanes 1 - 4: Merged signal (red and green). Green - **ab218512** observed at 12 kDa. Red - loading control, **ab181602**, observed at 37 kDa.

ab218512 was shown to specifically react with S100A4 in wild-type A549 cells as signal was lost in S100A4 knockout cell line **ab261865** (knockout cell lysate ab261674). Wild-type and S100A4 knockout samples were subjected to SDS-PAGE. Ab218512 and **ab181602** (Rabbit anti GAPDH loading control) were incubated overnight at 4°C at 1 ug/ml and 1/20000 dilution respectively. Blots were developed 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 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human S100A4 knockout A549 cell lysate (ab261674)

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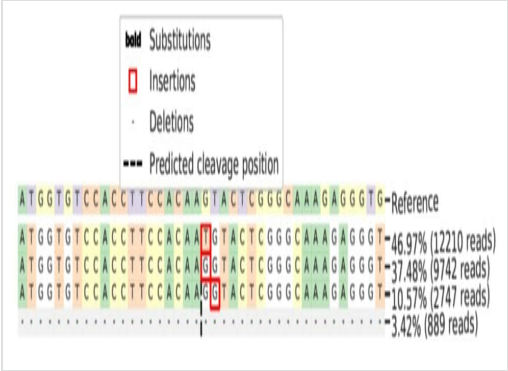
Lane 2: S100A4 knockout A549 (Human lung carcinoma cell line) whole cell lysate 20 ug

Lane 3: A-375 (Human malignant melanoma cell line) whole cell lysate 20 ug

Lane 4: HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate 20 ug

Lanes 1 - 4: Merged signal (red and green). Green - **ab218511** observed at 12 kDa. Red - loading control, **ab181602**, observed at 37 kDa.

ab218511 was shown to specifically react with S100A4 in wild-type A549 cells as signal was lost in S100A4 knockout cell line **ab261865** (knockout cell lysate ab261674). Wild-type and S100A4 knockout samples were subjected to SDS-PAGE. Ab218511 and **ab181602** (Rabbit anti GAPDH loading control) were incubated overnight at 4°C at 1 ug/ml and 1/20000 dilution respectively. Blots were developed 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 1/20000 dilution for 1 hour at room temperature before imaging.



Next Generation Sequencing - Human S100A4 knockout A549 cell lysate (ab261674)

Knockout achieved by CRISPR/Cas9; X = 1 bp insertion; Frameshift = 95%

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