abcam

Product datasheet

Human ASS1 knockout HeLa cell lysate ab257143

4 Images

Overview

Product name	Human ASS1 knockout HeLa cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon 3 and Insertion of the selection cassette in exon 3.
Passage number	<20
Knockout validation	Sanger Sequencing, 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

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

Components	1 kit
ab260139 - Human ASS1 knockout HeLa cell lysate	1 x 100µg
ab255552 - Human wild-type HeLa cell lysate	1 x 100µg

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

Target

Pathway Amino-acid biosynthesis; L-arginine biosynthesis; L-arginine from L-ornithine and carbamoyl phosphate: step 2/3.
Nitrogen metabolism; urea cycle; (N(omega)-L-arginino)succinate from L-aspartate and L-citrulline: step 1/1.

Involvement in disease Defects in ASS1 are the cause of citrullinemia type 1 (CTLN1) [MIM:215700]. Citrullinemia belongs to the urea cycle disorders. It is an autosomal recessive disease characterized primarily by elevated serum and urine citrulline levels. Ammonia intoxication is another manifestation. CTLN1 usually manifests in the first few days of life. Affected infants appear normal at birth, but as ammonia builds up in the body they present symptoms such as lethargy, poor feeding, vomiting, seizures and loss of consciousness. Less commonly, a milder CTLN1 form can develop later in childhood or adulthood.

Sequence similarities Belongs to the argininosuccinate synthase family. Type 1 subfamily.

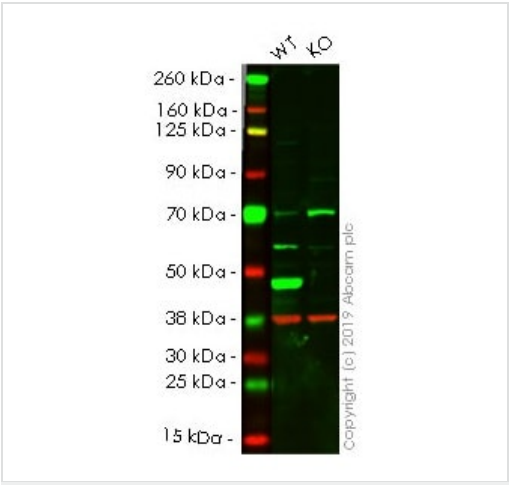
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257143 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



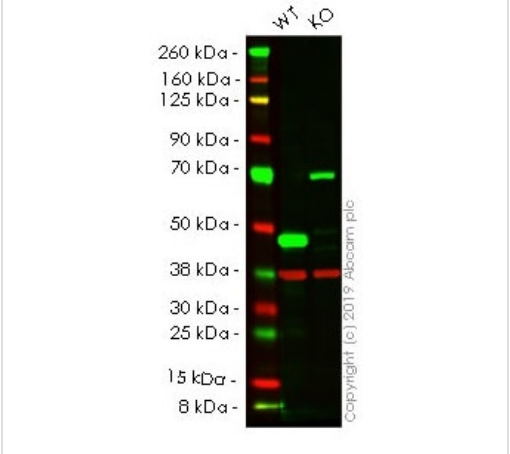
Western blot - Human ASS1 knockout HeLa cell lysate (ab257143)

Lane 1: Wild-type HeLa cell lysate (20µg)

Lane 2: ASS1 knockout HeLa cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - **ab170952** observed at 47 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab170952 Anti-ASS1 antibody [EPR12398] was shown to specifically react with ASS1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab264989** (knockout cell lysate ab257143) was used. Wild-type and ASS1 knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab170952** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 dilution 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 ASS1 knockout HeLa cell lysate (ab257143)

Lane 1: Wild-type HeLa cell lysate (20µg)

Lane 2: ASS1 knockout HeLa cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - **ab170900** observed at 47 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab170900 Anti-ASS1 antibody [EPR12399(B)] - C-terminal was shown to specifically react with ASS1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab264989** (knockout cell lysate ab257143) was used. Wild-type and ASS1 knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab170900** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 dilution 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 ASS1 knockout HeLa cell lysate (ab257143)

Allele-1: 1 bp insertion in exon 3

Sanger Sequencing - Human ASS1 knockout HeLa cell lysate (ab257143)

Allele-2: Insertion of the selection cassette in exon 3

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