# abcam

#### Product datasheet

## Anti-ASS1 antibody [EPR12398] ab170952





#### ★★★★★ 1 Abreviews 8 References 20 Images

#### Overview

**Product name** Anti-ASS1 antibody [EPR12398]

**Description** Rabbit monoclonal [EPR12398] to ASS1

**Host species** Rabbit

**Tested applications** Suitable for: Flow Cyt (Intra), WB, ICC/IF, IP, IHC-P

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Cow

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HAP1, HeLa, HepG2 cell lysates. Human fetal kidney and liver tissue lysates. Mouse liver

and kidney lysates. Rat liver lysate. ICC/IF: MCF7 and HeLa cells. IHC-P: Human kidney, ureter

tissue. Mouse kidney tissue. Flow Cyt (intra): HeLa cells. IP: HeLa cells.

**General notes** The rat recommendation is based on the WB results. This antibody may not be suitable for IHC

with rat samples.

This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

#### **Properties**

**Form** Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

**Purity** Protein A purified

Clonality Monoclonal Clone number EPR12398

Isotype ΙqG

#### **Applications**

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab170952 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
Flow Cyt (Intra)		1/100. For unpurified use at 1/500 - 1/1000. <u>ab172730</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB		1/20000. Predicted molecular weight: 47 kDa.  For unpurified use at 1/1000 - 1/10000.
ICC/IF		1/50 - 1/100.
IP		1/10 - 1/100.
IHC-P	*****(1)	1/4000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. See IHC antigen retrieval protocols.  For unpurified use at 1/250 - 1/500.

Target
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**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.

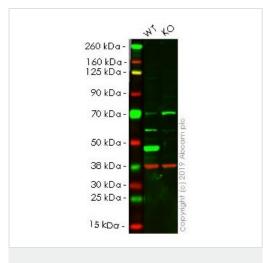
Involvement in disease Defects in ASS1 are the cause of citrullinemia type 1 (CTLN1) [MIM:215700]. Citrullinemia

> 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.

#### **Images**



Western blot - Anti-ASS1 antibody [EPR12398] (ab170952)

**All lanes :** Anti-ASS1 antibody [EPR12398] (ab170952) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: ASS1 knockout HeLa cell lysate

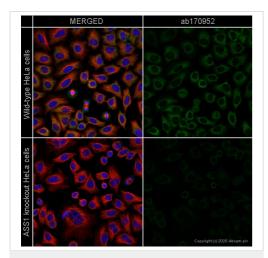
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

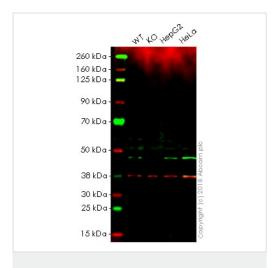
**Predicted band size:** 47 kDa **Observed band size:** 47 kDa

**Lanes 1-2:** Merged signal (red and green). Green - ab170952 observed at 47 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab170952 was shown to 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 HeLa and ASS1 knockout HeLa cell lysates 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) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-ASS1 antibody [EPR12398] (ab170952)



Western blot - Anti-ASS1 antibody [EPR12398] (ab170952)

ab170952 staining ASS1 in wild-type HeLa cells (top panel) and ASS1 knockout HeLa cells (ab264989) (bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab170952 at 1/100 dilution and ab7291 (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit lgG (Alexa Fluor<sup>®</sup> 488) (ab150081) at 2 μg/ml (shown in green) and a goat secondary antibody to mouse lgG (Alexa Fluor<sup>®</sup> 594) (ab150120) at 2 μg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).

**All lanes :** Anti-ASS1 antibody [EPR12398] (ab170952) at 1/20000 dilution

Lane 1: Wild-type HAP1 whole cell lysate

Lane 2: ASS1 knockout HAP1 whole cell lysate

Lane 3 : HepG2 whole cell lysate

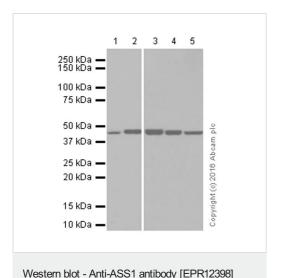
Lane 4 : HeLa whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 47 kDa Observed band size: 47 kDa

**Lanes 1 - 4:** Merged signal (red and green). Green - ab170952 observed at 47 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab170952 was shown to recognize ASS1 in wild-type HAP1 cells as signal was lost at the expected MW in ASS1 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and ASS1 knockout samples were subjected to SDS-PAGE. Ab170952 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/20000 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/10000



(ab170952)

dilution for 1 hour at room temperature before imaging.

All lanes : Anti-ASS1 antibody [EPR12398] (ab170952) at 0.05  $\mu$ g/ml (Purified)

**Lane 1 :** HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 2: Human fetal liver lysate

Lane 3 : Mouse liver lysate

Lane 4: Rat liver lysate

Lane 5: Mouse kidney lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

**Predicted band size:** 47 kDa **Observed band size:** 47 kDa

Blocking and diluting buffer: 5% NFDM/TBST

ab170952 (purified) at 1:60 dilution (5ug) immunoprecipitating ASS1 in HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate.

Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10ug

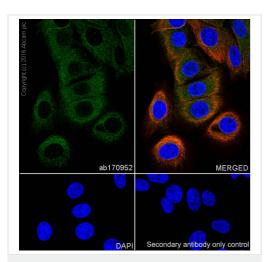
Lane 2 (+): ab170952 & HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

**Lane 3 (-):** Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab170952 in HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1:1000 dilution.

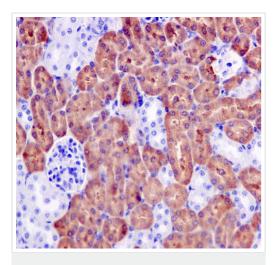
Blocking and diluting buffer: 5% NFDM/TBST.

Immunoprecipitation - Anti-ASS1 antibody [EPR12398] (ab170952)



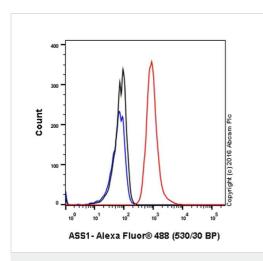
Immunocytochemistry/ Immunofluorescence - Anti-ASS1 antibody [EPR12398] (ab170952)

Immunocytochemistry/ Immunofluorescence analysis of MCF7 (Human breast adenocarcinoma cell line) cells labeling ASS1 with Purified ab170952 at 1:100 dilution (10.2 $\mu$ g/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) 1:200 (2.5  $\mu$ g/ml). **ab150077** Goat anti rabbit lgG(Alexa Fluor<sup>®</sup> 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



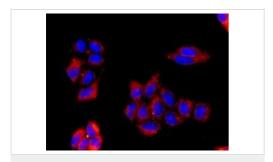
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ASS1 antibody
[EPR12398] (ab170952)

ab170952 showing +ve staining in Mouse kidney tissue.



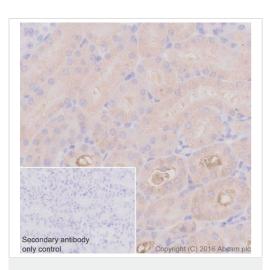
Flow Cytometry (Intracellular) - Anti-ASS1 antibody [EPR12398] (ab170952)

Intracellular Flow Cytometry analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling ASS1 with purified ab170952 at 1/100 dilution (10 ug/ml) (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit lgG (Alexa Fluor  $^{\mbox{\it R}}$  488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal lgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunocytochemistry/ Immunofluorescence - Anti-ASS1 antibody [EPR12398] (ab170952)

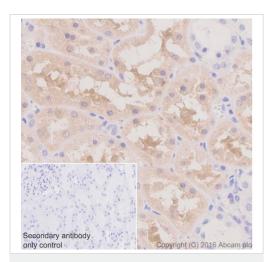
Immunofluorescent analysis of HeLa cells labeling ASS1 using ab170952 at 1/50 dilution (red). DAPI nuclear staining (blue).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ASS1 antibody
[EPR12398] (ab170952)

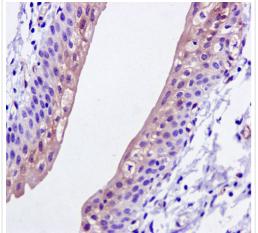
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Mouse kidney tissue sections labeling ASS1 with Purified ab170952 at 1:4000 dilution (0.25 µg/ml). Heat mediated antigen retrieval was performed using citrate Buffer, PH6. Tissue was counterstained with Hematoxylin. <a href="mailto:ab97051"><u>ab97051</u></a> Goat Anti-Rabbit IgG H&L (HRP)

secondary antibody was used at 1:500 dilution. PBS instead of the primary antibody was used as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ASS1 antibody
[EPR12398] (ab170952)

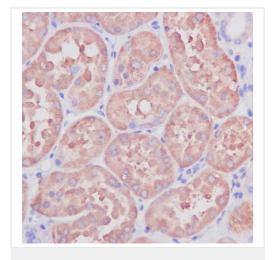
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human kidney tissue sections labeling ASS1 with Purified ab170952 at 1:4000 dilution (0.25 µg/ml). Heat mediated antigen retrieval was performed using citrate Buffer, PH6. Tissue was counterstained with Hematoxylin. <a href="mailto:ab97051">ab97051</a> Goat Anti-Rabbit IgG H&L (HRP) secondary antibody was used at 1:500 dilution. PBS instead of the primary antibody was used as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ASS1 antibody

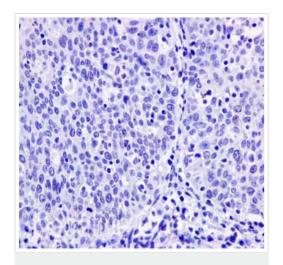
[EPR12398] (ab170952)

ab170952 showing +ve staining in Human normal ureter tissue.



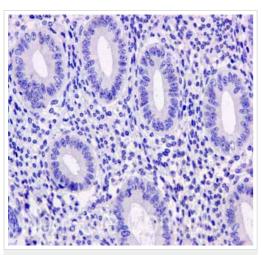
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ASS1 antibody
[EPR12398] (ab170952)

Immunohistochemical analysis of paraffin-embedded Human kidney tissue labeling ASS1 with ab170952 at 1/250 dilution.



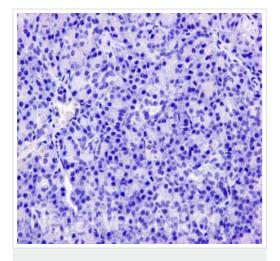
ab170952 showing -ve staining in Human cervical carcinoma tissue.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ASS1 antibody
[EPR12398] (ab170952)



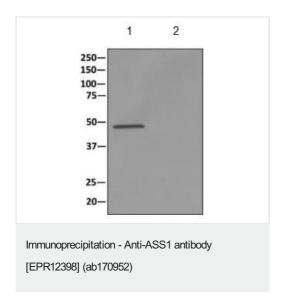
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ASS1 antibody
[EPR12398] (ab170952)

ab170952 showing -ve staining in Human normal uterus tissue.



ab170952 showing -ve staining in Human normal pancreas tissue.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ASS1 antibody
[EPR12398] (ab170952)

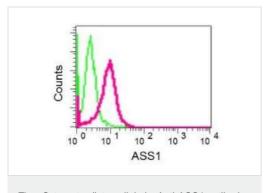


Secondary antibody used is HRP-conjugated anti-rabbit IgG preferentially detecting the non-reduced form of rabbit IgG.

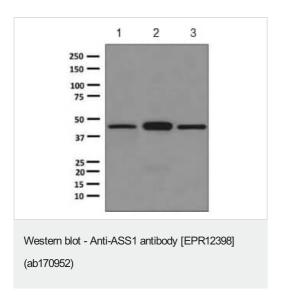
**All lanes :** Anti-ASS1 antibody [EPR12398] (ab170952) at 1/10 dilution

Lane 1: Human fetal liver tissue lysate at 10 µg

Lane 2: PBS



Flow Cytometry (Intracellular) - Anti-ASS1 antibody [EPR12398] (ab170952) Intracellular flow cytometric analysis of permeabilized Hela cells labeling ASS1 using ab170952 at 1/500 dilution (red) or a rabbit lgG negative (green).



**All lanes**: Anti-ASS1 antibody [EPR12398] (ab170952) at 1/1000 dilution

Lane 1: HeLa cell lysate

Lane 2 : Fetal liver tissue lysate

Lane 3: Fetal kidney tissue lysate

Lysates/proteins at 10 µg per lane.

#### Secondary

All lanes: Goat anti-rabbit HRP at 1/2000 dilution

Predicted band size: 47 kDa



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