abcam

Product datasheet

Anti-ACSS2 antibody ab66038

KO VALIDATED

3 Abreviews 8 References 4 Images

Overview

Product name	Anti-ACSS2 antibody
Description	Rabbit polyclonal to ACSS2
Host species	Rabbit
Specificity	Replenishment batches of our polyclonal antibody, ab66038 are tested in WB. Previous batches were additionally validated in IHC-P and IP. These applications are still expected to work and are covered by our Abpromise guarantee. You may also be interested in our alternative recombinant antibody, **ab133664**.
Tested applications	**Suitable for:** WB, IHC-P, IP **Unsuitable for:** ICC/IF
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat
Immunogen	Synthetic peptide corresponding to Human ACSS2 aa 650 to the C-terminus (C terminal) conjugated to keyhole limpet haemocyanin. (Peptide available as **ab71894**, **ab71895**)
Positive control	This antibody gave a positive signal in Human Liver and Human Colon Tissue lysates and in the following whole cell lysates: HepG2; U-87 MG; Caco 2.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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 or -80°C. Avoid freeze / thaw cycle.

Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab66038 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

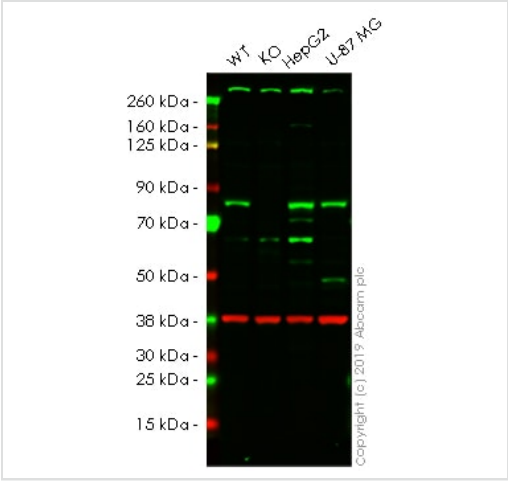
Application	Abreviews	Notes
WB	★★★★☆ (1)	Use a concentration of 1 µg/ml. Detects a band of approximately 79 kDa (predicted molecular weight: 79 kDa).
IHC-P		Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
IP	★★★★★ (1)	Use at an assay dependent concentration.

Application notes Is unsuitable for ICC/IF.

Target

Function	Activates acetate so that it can be used for lipid synthesis or for energy generation.
Sequence similarities	Belongs to the ATP-dependent AMP-binding enzyme family.
Cellular localization	Cytoplasm.

Images



Western blot - Anti-ACSS2 antibody (ab66038)

All lanes : Anti-ACSS2 antibody (ab66038) at 1 µg/ml

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : ACSS2 knockout HAP1 whole cell lysate
Lane 3 : HepG2 whole cell lysate
Lane 4 : U-87 MG whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 79 kDa
Observed band size: 80 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab66038 observed at 80 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab66038 was shown to recognize ACSS2 in wild-type HAP1 cells as signal was lost at the expected MW in ACSS2 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and ACSS2 knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% Milk. Ab66038 and **ab8245** (Mouse 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-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 - Anti-ACSS2 antibody (ab66038)

All lanes : Anti-ACSS2 antibody (ab66038) at 1 µg/ml

Lane 1 : Human liver tissue lysate - total protein (**ab29889**)
Lane 2 : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate
Lane 3 : U-87 MG (Human glioblastoma astrocytoma) Whole Cell Lysate
Lane 4 : Human colon tissue lysate - total protein (**ab30051**)
Lane 5 : Caco-2 whole cell lysate (**ab3950**)

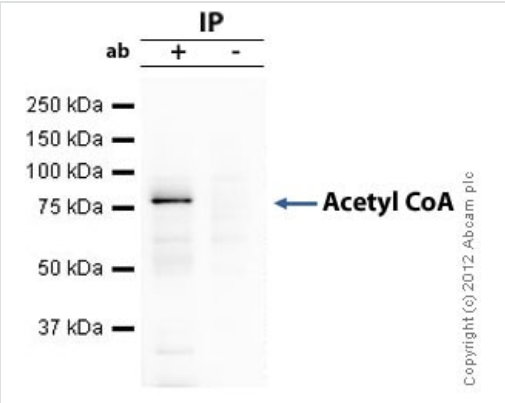
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Performed under reducing conditions.

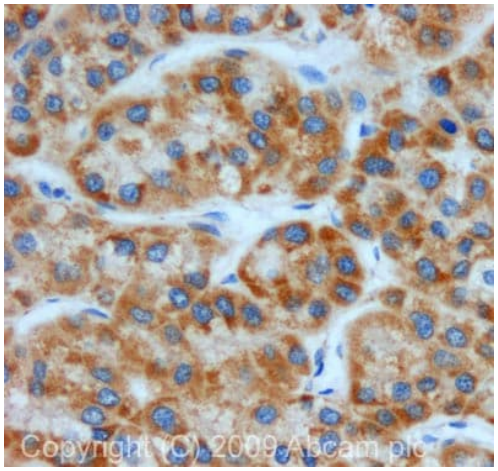
Predicted band size: 79 kDa
Observed band size: 79 kDa
Additional bands at: 100 kDa, 38 kDa, 45 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 30 seconds



Immunoprecipitation - Anti-ACSS2 antibody (ab66038)

ACSS2 was immunoprecipitated using 0.5mg HepG2 whole cell extract, 5µg of Rabbit polyclonal to ACSS2 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).
The antibody was incubated under agitation with Protein G beads for 10min, HepG2 whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70^{o}C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab66038.
Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (**ab99697**).
Band: 79kDa: ACSS2.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ACSS2 antibody (ab66038)

IHC image of ACSS2 staining in human liver carcinoma formalin fixed paraffin embedded tissue section, performed on a Leica Bond^{TM} system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab66038, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

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