

Anti-ACAT1 Antibody Picoband™

Catalog Number: A02008-1

About ACAT1

Acetyl-CoA acetyltransferase, mitochondrial, also known as acetoacetyl-CoA thiolase, is an enzyme that in humans is encoded by the ACAT1 (Acetyl-Coenzyme A acetyltransferase 1) gene. This gene encodes a mitochondrially localized enzyme that catalyzes the reversible formation of acetoacetyl-CoA from two molecules of acetyl-CoA. Defects in this gene are associated with 3-ketothiolase deficiency, an inborn error of isoleucine catabolism characterized by urinary excretion of 2-methyl-3-hydroxybutyric acid, 2-methylacetoacetic acid, tiglylglycine, and butanone.

Overview

Product Name	Anti-ACAT1 Antibody Picoband™
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-ACAT1 Antibody Picoband™ catalog # A02008-1. Tested in ELISA, IF, IHC, ICC, WB, Flow Cytometry applications. This antibody reacts with Human, Mouse, Rat.
Application	ELISA, Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl, 0.2 mg Na ₂ HPO ₄ .
Storage Instructions	At -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freezing and thawing.
Host	Rabbit
Uniprot ID	P24752

Technical Details

Immunogen	E.coli-derived human ACAT1 recombinant protein (Position: S10-Q404).
Predicted Reactive Species	Human
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
Purification	Immunogen affinity purified.

Suggested Dilutions

Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.

If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.

Some PubMed article(s) citing the expression level of this target are as follows:

Boster Bio's internal QC testing used:

Western blot, 0.25-0.5 µg/ml, Human, Mouse, Rat

Immunohistochemistry(Paraffin-embedded Section), 2-5 µg/ml, Human

Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human

Flow Cytometry (Fixed), 1-3 µg/1x10⁶ cells, Human

Direct ELISA, 0.1-0.5 µg/ml, Human

Anti-ACAT1 Antibody Picoband™ (A02008-1) Images

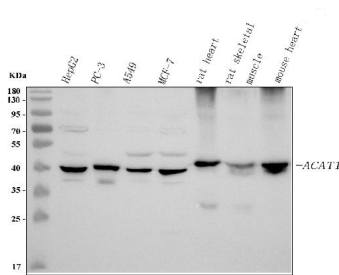


Figure 1. Western blot analysis of ACAT1 using anti-ACAT1 antibody (A02008-1).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: human HepG2 whole cell lysates,

Lane 2: human PC-3 whole cell lysates,

Lane 3: human A549 whole cell lysates,

Lane 4: human MCF-7 whole cell lysates,

Lane 5: rat heart tissue lysates,

Lane 6: rat skeletal muscle tissue lysates,

Lane 7: mouse heart tissue lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes.

Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-ACAT1 antigen affinity purified polyclonal antibody (Catalog # A02008-1) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for ACAT1 at approximately 40 kDa. The expected band size for ACAT1 is at 40 kDa.

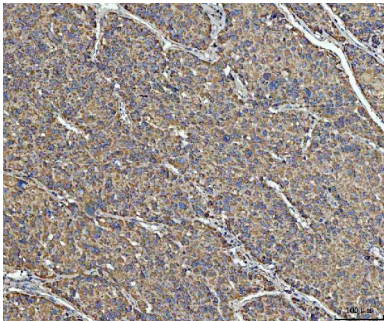


Figure 2. IHC analysis of ACAT1 using anti-ACAT1 antibody (A02008-1).

ACAT1 was detected in a paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-ACAT1 Antibody (A02008-1) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

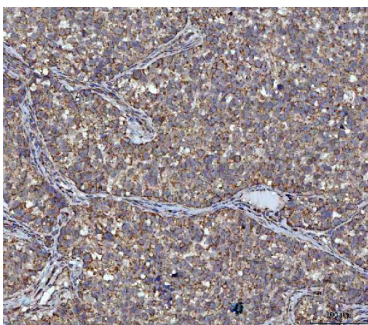


Figure 3. IHC analysis of ACAT1 using anti-ACAT1 antibody (A02008-1).

ACAT1 was detected in a paraffin-embedded section of human non-small cell lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-ACAT1 Antibody (A02008-1) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog

SV0002) with DAB as the chromogen.

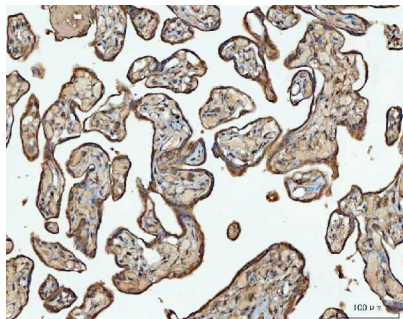


Figure 4. IHC analysis of ACAT1 using anti-ACAT1 antibody (A02008-1).

ACAT1 was detected in a paraffin-embedded section of human placenta tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-ACAT1 Antibody (A02008-1) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

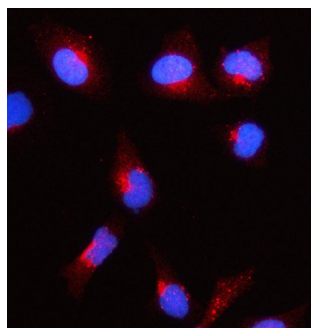


Figure 5. IF analysis of ACAT1 using anti-ACAT1 antibody (A02008-1).

ACAT1 was detected in an immunocytochemical section of HELA cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 ug/mL rabbit anti-ACAT1 Antibody (A02008-1) overnight at 4°C. Cy3 Conjugated Goat Anti-Rabbit IgG (BA1032) was used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

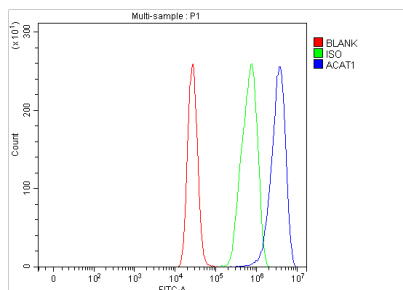


Figure 6. Flow Cytometry analysis of MCF-7 cells using anti-ACAT1 antibody (A02008-1).

Overlay histogram showing MCF-7 cells stained with A02008-1 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-ACAT1 Antibody (A02008-1, 1 ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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