

Anti-ATP citrate lyase ACLY Antibody Picoband™ (monoclonal, 5I2)

Catalog # ABO14851

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P53396
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-ATP citrate lyase ACLY Antibody Picoband™ (monoclonal, 5I2) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500 µg/ml.

Additional Information

Gene ID	47
Other Names	ATP-citrate synthase, 2.3.3.8, ATP-citrate (pro-S-)-lyase, ACL, Citrate cleavage enzyme, ACLY
Calculated MW	120839
Application Details	Western blot, 0.1-0.5 µg/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml Immunocytochemistry/Immunofluorescence, 2 µg/ml, Human Flow Cytometry, 1-3 µg/1x10 ⁶ cells
Subcellular Localization	Cytoplasm.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na ₂ HPO ₄ , 0.05mg Na ₃ .
Clone Names Immunogen	Clone: 5I2 E. coli-derived human ATP citrate lyase recombinant protein (Position: M1-I180). Human ATP citrate lyase shares 95% amino acid (aa) sequence identity with both mouse and rat ATP citrate lyase.
Cross Reactivity	No cross-reactivity with other proteins.

Storage	Store 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 freeze-thaw cycles.
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Protein Information

Name	ACLY
Function	Catalyzes the cleavage of citrate into oxaloacetate and acetyl-CoA, the latter serving as common substrate in multiple biochemical reactions in protein, carbohydrate and lipid metabolism.
Cellular Location	Cytoplasm, cytosol.

Background

ATP citrate lyase, also known as ACLY, is an enzyme that in animals represents an important step in fatty acid biosynthesis. ATP citrate lyase is the primary enzyme responsible for the synthesis of cytosolic acetyl-CoA in many tissues. The enzyme is a tetramer of apparently identical subunits. The product, acetyl-CoA, in animals serves several important biosynthetic pathways, including lipogenesis and cholesterol synthesis. It is activated by insulin. In nervous tissue, ATP citrate-lyase may be involved in the biosynthesis of acetylcholine. In plants, ATP citrate lyase generates the acetyl-CoA for cytosolically-synthesized metabolites.

Images

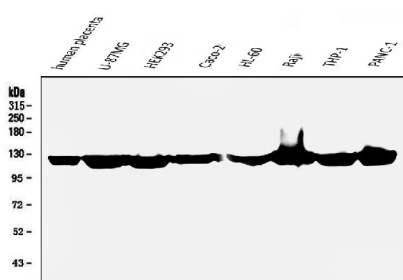


Figure 1. Western blot analysis of ATP citrate lyase using anti-ATP citrate lyase antibody (M02372-1).

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 50ug of sample under reducing conditions.

Lane 1: human placenta tissue lysates,

Lane 2: U-87MG whole cell lysates,

Lane 3: HEK293 whole cell lysates,

Lane 4: Caco-2 whole cell lysates,

Lane 5: HL-60 whole cell lysates,

Lane 6: Raji whole cell lysates,

Lane 7: THP-1 whole cell lysates,

Lane 8: PANC-1 whole cell lysates.

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-ATP citrate lyase antigen affinity purified monoclonal antibody (Catalog # M02372-1) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse 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 # EK1001) with Tanon 5200 system. A specific band was detected for ATP citrate lyase at approximately 121KD. The expected band size for ATP citrate lyase is at 121KD.

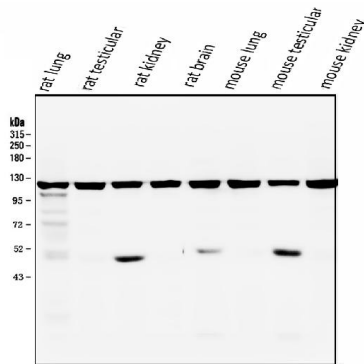


Figure 2. Western blot analysis of ATP citrate lyase using anti-ATP citrate lyase antibody (M02372-1). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions. Lane 1: rat lung tissue lysates, Lane 2: rat testicular tissue lysates, Lane 3: rat kidney tissue lysates, Lane 4: rat brain tissue lysates, Lane 5: mouse lung tissue lysates, Lane 6: mouse testicular tissue lysates, Lane 7: mouse kidney tissue lysates. After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-ATP citrate lyase antigen affinity purified monoclonal antibody (Catalog # M02372-1) at 0.5 μ g/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse 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 # EK1001) with Tanon 5200 system. A specific band was detected for ATP citrate lyase at approximately 121KD. The expected band size for ATP citrate lyase is at 121KD.

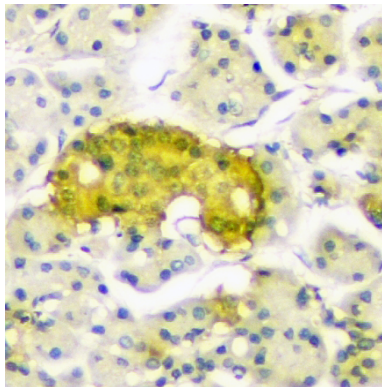


Figure 3. IHC analysis of ATP citrate lyase using anti-ATP citrate lyase antibody (M02372-1). ATP citrate lyase was detected in paraffin-embedded section of human pancreatic cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 μ g/ml mouse anti-ATP citrate lyase Antibody (M02372-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

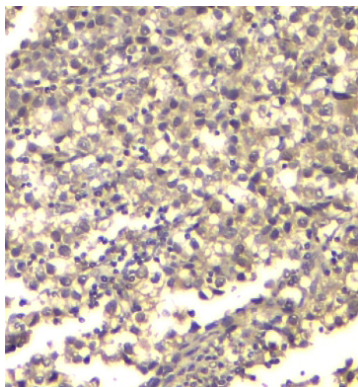
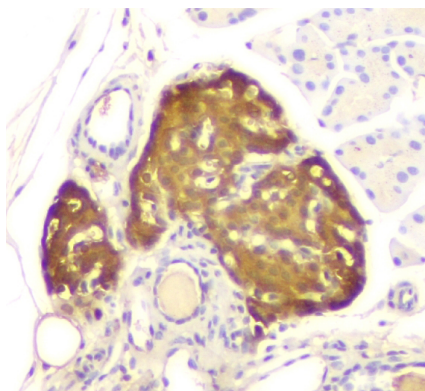


Figure 4. IHC analysis of ATP citrate lyase using anti-ATP citrate lyase antibody (M02372-1). ATP citrate lyase was detected in paraffin-embedded section of human testis cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 μ g/ml mouse anti-ATP citrate lyase Antibody (M02372-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

Figure 5. IHC analysis of ATP citrate lyase using anti-ATP citrate lyase antibody (M02372-1). ATP citrate lyase was detected in paraffin-embedded



section of mouse pancreas tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-ATP citrate lyase Antibody (M02372-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

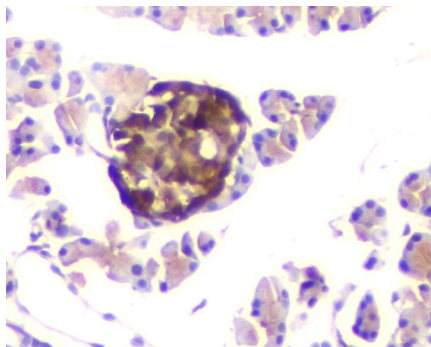


Figure 6. IHC analysis of ATP citrate lyase using anti-ATP citrate lyase antibody (M02372-1). ATP citrate lyase was detected in paraffin-embedded section of rat pancreas tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-ATP citrate lyase Antibody (M02372-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

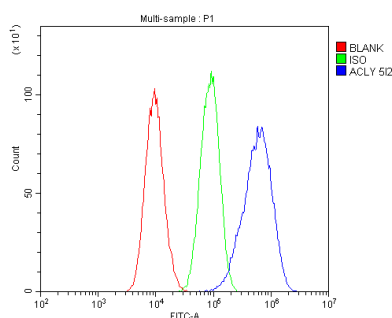


Figure 7. Flow Cytometry analysis of A549 cells using anti-ATP citrate lyase antibody (M02372-1). Overlay histogram showing A549 cells stained with M02372-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-ATP citrate lyase Antibody (M02372-1, 1 µg/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

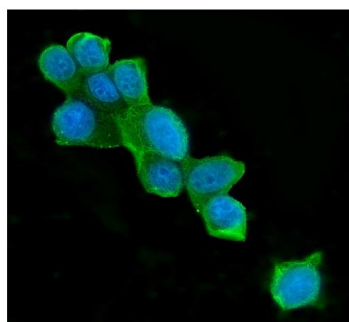


Figure 8. IF analysis of ATP citrate lyase using anti-ATP citrate lyase antibody (M02372-1). ATP citrate lyase was detected in immunocytochemical section of MCF7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-ATP citrate lyase Antibody (M02372-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 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.