Anti-ACY1 Antibody [15G2]

EM1901-86

Product Type:	Mouse monoclonal IgG2a, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, FC
Molecular Wt:	Predicted band size: 46 kDa
Clone number:	15G2

Description: Aminoacylase is involved in the regulation of the urea cycle. N-acetyl-L-glutamate is an allosteric activator of carbamoyl phosphate synthetase, a crucial enzyme that commits NH4+ molecules to the urea cycle. The urea cycle gets rid of excess ammonia (NH4+) in the body, a process that must be up-regulated during times of increased protein catabolism, as amino acid breakdown produces large amounts of NH4+. When amino acid catabolism increases, N-Acetylglutamate synthase is up-regulated, producing more N-acetyl-L-glutamate, which up-regulates carbamoyl phosphate synthetase and allows it to dispose of the excess NH4+ from catabolism.Aminoacylase is up-regulated during times of nutrient deficit or starvation, causing N-acetyl-L-glutamate breakdown, which down-regulates carbamoyl phosphate synthetase and the rest of the urea cycle. This response is evolutionarily advantageous, since a nutrient deficit means there isn't as much NH4+ that needs to be disposed of and since the body wants to salvage as many amino acids as it can.

Immunogen: Recombinant protein within Human ACY1 aa 55-256 / 408.

Positive control: K-562 cell lysate, HepG2 cell lysate, PC-3M cell lysate, human liver tissue lysate, human kidney tissue lysate, mouse liver tissue lysate, mouse kidney tissue lysate, rat liver tissue lysate, rat kidney tissue lysate, human kidney tissue, mouse kidney tissue, rat kidney tissue, SHSY5Y.

Subcellular location: Cytoplasm.

Database links: SwissProt: Q03154 Human | Q99JW2 Mouse | Q6AYS7 Rat

Recommended Dilutions:

WB	1:500-1:1,000
IHC-P	1:2,000
FC	1:50-1:100

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

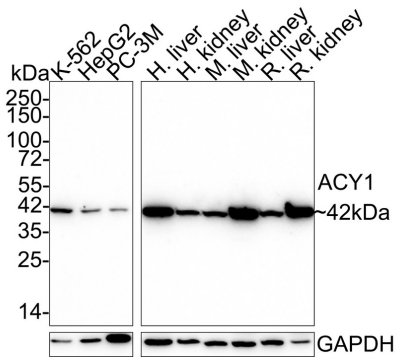

Fig1: Western blot analysis of ACY1 on different lysates with Mouse anti-ACY1 antibody (EM1901-86) at 1/1,000 dilution.

Lane 1: K-562 cell lysate (20 µg/Lane)
Lane 2: HepG2 cell lysate (20 µg/Lane)
Lane 3: PC-3M cell lysate (20 µg/Lane)
Lane 4: Human liver tissue lysate (40 µg/Lane)
Lane 5: Human kidney tissue lysate (40 µg/Lane)
Lane 6: Mouse liver tissue lysate (40 µg/Lane)
Lane 7: Mouse kidney tissue lysate (40 µg/Lane)
Lane 8: Rat liver tissue lysate (40 µg/Lane)
Lane 9: Rat kidney tissue lysate (40 µg/Lane)

Predicted band size: 46 kDa
Observed band size: 42 kDa

Exposure time: 3 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (EM1901-86) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

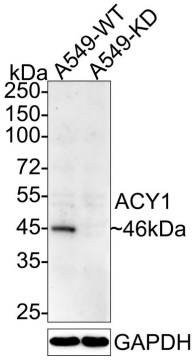

Fig2: Western blot analysis of ACY1 on different lysates with Mouse anti-ACY1 antibody (EM1901-86) at 1/1,000 dilution.

Lane 1: A549-si NT cell lysate
Lane 2: A549-si ACY1 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 46 kDa
Observed band size: 46 kDa

Exposure time: 3 minutes; ECL: K1802;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (EM1901-86) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

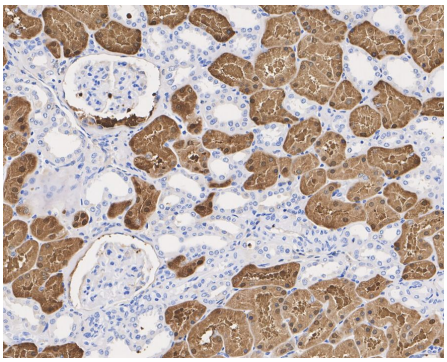

Fig3: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Mouse anti-ACY1 antibody (EM1901-86) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM1901-86) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

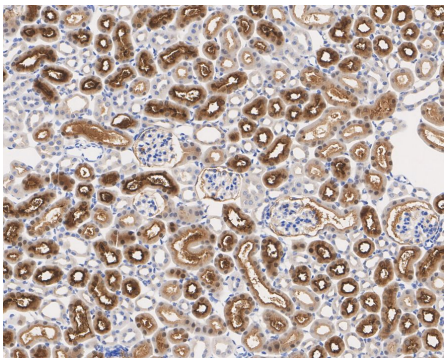

Fig4: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Mouse anti-ACY1 antibody (EM1901-86) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM1901-86) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

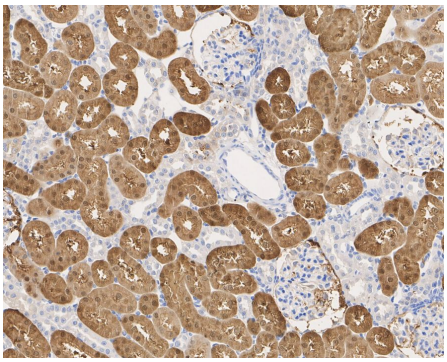

Fig5: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Mouse anti-ACY1 antibody (EM1901-86) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM1901-86) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

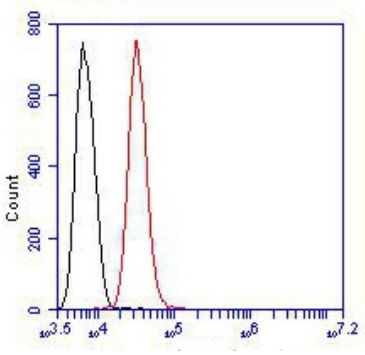

Fig6: Flow cytometric analysis of ACY1 was done on SHSY5Y cells. The cells were fixed, permeabilized and stained with the primary antibody (EM1901-86, 1/100) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa FluorTM488 Goat anti-Mouse IgG IgG Secondary antibody at 1/500 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Lindner H.A.et.al.Essential roles of zinc ligation and enzyme dimerization for catalysis in the aminoacylase-1/M20 family.J. Biol. Chem. 278:44496-44504(2003).