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Anti-PCK2 Antibody Picoband™ (monoclonal, 3F7)

Catalog # ABO14980

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	Q16822
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse, Monkey
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-PCK2 Antibody Picoband™ (monoclonal, 3F7) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Monkey, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	5106
Other Names	Phosphoenolpyruvate carboxykinase [GTP], mitochondrial, PEPCK-M, 4.1.1.32, Phosphoenolpyruvate carboxykinase 2, mitochondrial, mtPCK2, PCK2 (HGNC:8725), PEPCK2
Calculated MW	70699
Application Details	Western blot, 0.1-0.25 □g/ml, Human, Mouse, Rat, Monkey Immunohistochemistry (Paraffin-embedded Section), 2-5 □g/ml, Human Immunocytochemistry/Immunofluorescence, 5 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Clone Names	Clone: 3F7
Immunogen	E.coli-derived human PCK2 recombinant protein (Position: M1-M640).
Purification	Immunogen affinity purified.
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.

Protein Information

Name	PCK2 (HGNC:8725)
Synonyms	PEPCK2
Function	Mitochondrial phosphoenolpyruvate carboxykinase that catalyzes the conversion of oxaloacetate (OAA) to phosphoenolpyruvate (PEP), the rate-limiting step in the metabolic pathway that produces glucose from lactate and other precursors derived from the citric acid cycle (PubMed:28955899). Can play an active role in glyceroneogenesis and gluconeogenesis (PubMed:28955899). In lipogenic cells such as adipocytes, it generates PEP via the mitochondrial pyruvate-to-PEP bypass, driving the PEP flux required for cytosolic glycerol 3- phosphate and triglyceride synthesis (By similarity). Also acts as a serine/threonine-protein kinase: phosphorylates and activates ACSL4, thereby promoting ferroptosis (PubMed:38720107).
Cellular Location	Mitochondrion
Tissue Location	Widely expressed..

Background

Phosphoenolpyruvate carboxykinase 2, mitochondrial (PCK2, PEPCK-M), is an isozyme of phosphoenolpyruvate carboxykinase (PCK, PEPCK) that in humans is encoded by the PCK2 gene. It is mapped to 14q11.2-q12. This gene encodes a mitochondrial enzyme that catalyzes the conversion of oxaloacetate to phosphoenolpyruvate in the presence of guanosine triphosphate (GTP). A cytosolic form of this protein is encoded by a different gene and is the key enzyme of gluconeogenesis in the liver. Alternatively spliced transcript variants have been described.

Images

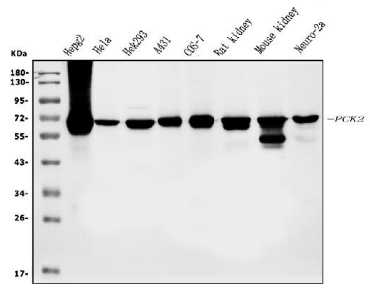


Figure 1. Western blot analysis of PCK2 using anti-PCK2 antibody (M04772-2).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human HEPG2 whole cell lysates,
Lane 2: human Hela whole cell lysates,
Lane 3: human HEK293 whole cell lysates,
Lane 4: human A431 whole cell lysates,
Lane 5: monkey COS-7 whole cell lysates,
Lane 6: rat kidney tissue lysates,
Lane 7: mouse kidney tissue lysates,
Lane 8: mouse Neuro-2a 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-PCK2 antigen affinity purified monoclonal antibody (Catalog # M04772-2) at 0.25 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 1:10000 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 PCK2 at approximately 71KD. The expected band size

forPCK2 is at 71KD.

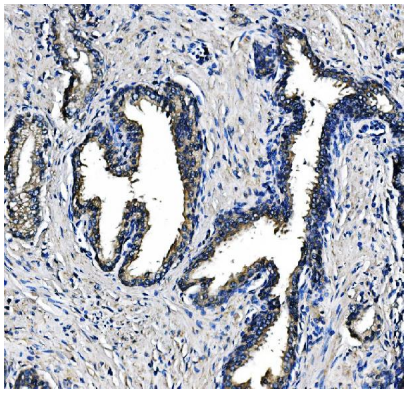


Figure 2. IHC analysis of PCK2 using anti-PCK2 antibody (M04772-2).
PCK2 was detected in paraffin-embedded section of human prostate cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-PCK2 Antibody (M04772-2) 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 Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

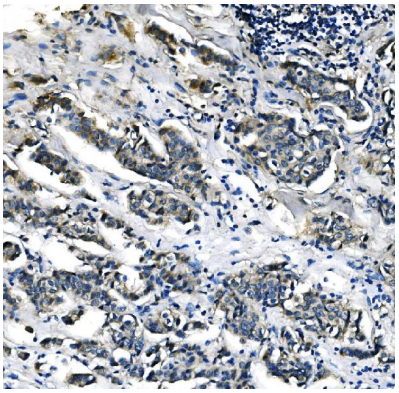


Figure 3. IHC analysis of PCK2 using anti-PCK2 antibody (M04772-2).
PCK2 was detected in paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-PCK2 Antibody (M04772-2) 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 Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

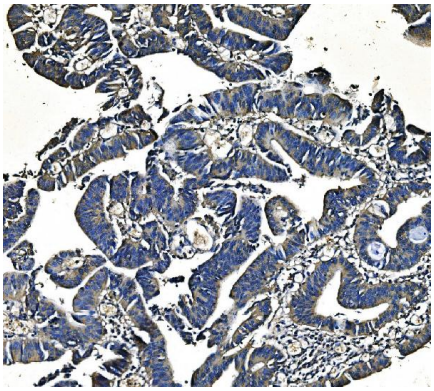


Figure 4. IHC analysis of PCK2 using anti-PCK2 antibody (M04772-2).
PCK2 was detected in paraffin-embedded section of human retcal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-PCK2 Antibody (M04772-2) 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 Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

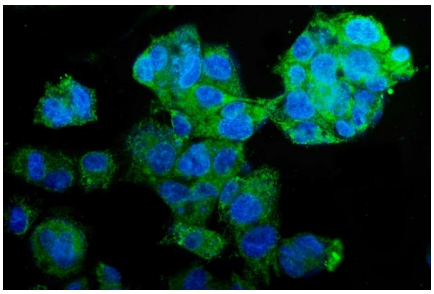


Figure 5. IF analysis of PCK2 using anti-PCK2 antibody (M04772-2).
PCK2 was detected in immunocytochemical section of HEPG2 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-PCK2 Antibody (M04772-2) 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.

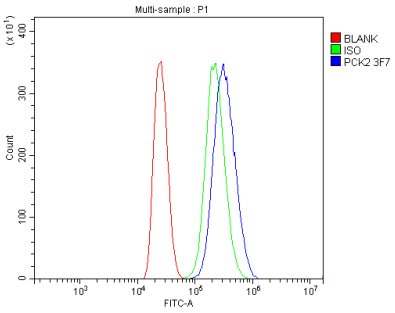


Figure 6. Flow Cytometry analysis of MCF-7 cells using anti-PCK2 antibody (M04772-2).
Overlay histogram showing MCF-7 cells stained with M04772-2 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-PCK2 Antibody (M04772-2, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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