

Anti-PCK1/PEPC Antibody Picoband™

Catalog Number: A02022-3

About PCK1

This gene is a main control point for the regulation of gluconeogenesis. The cytosolic enzyme encoded by this gene, along with GTP, catalyzes the formation of phosphoenolpyruvate from oxaloacetate, with the release of carbon dioxide and GDP. The expression of this gene can be regulated by insulin, glucocorticoids, glucagon, cAMP, and diet. Defects in this gene are a cause of cytosolic phosphoenolpyruvate carboxykinase deficiency. A mitochondrial isozyme of the encoded protein also has been characterized.

Overview

Product Name	Anti-PCK1/PEPC Antibody Picoband™
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-PCK1/PEPC Antibody Picoband™ catalog # A02022-3. Tested in ELISA, Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	ELISA, Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.01mg NaN3.
Storage Instructions	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.
Host	Rabbit
Uniprot ID	P35558

Technical Details

Immunogen	E.coli-derived human PCK1/PEPC recombinant protein (Position: M1-M622).
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 ug/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, 0.25-0.5ug/ml, Human, Mouse, Rat

Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Mouse, Rat

Immunocytochemistry/Immunofluorescence, 4ug/ml, Human

Flow Cytometry, 1-3ug/1x10⁶ cells, Human

Direct ELISA, 0.1-0.5ug/ml, Human

Anti-PCK1/PEPC Antibody Picoband™ (A02022-3) Images

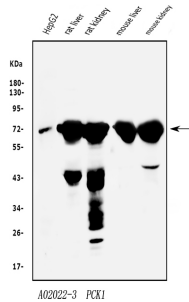


Figure 1. Western blot analysis of PCK1/PEPC using anti-PCK1/PEPC antibody (A02022-3).

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: rat liver tissue lysates,

Lane 3: rat kidney tissue lysates,

Lane 4: mouse liver tissue lysates,

Lane 5: 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 rabbit anti-PCK1/PEPC antigen affinity purified polyclonal antibody (Catalog # A02022-3) 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 1:10000 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 PCK1/PEPC at approximately 69KD. The expected band size for PCK1/PEPC is at 69KD.

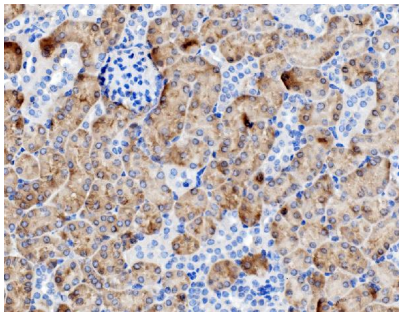


Figure 2. IHC analysis of PCK1/PEPC using anti-PCK1/PEPC antibody (A02022-3).

PCK1/PEPC was detected in paraffin-embedded section of mouse kidney 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 1ug/ml rabbit anti-PCK1/PEPC Antibody (A02022-3) overnight at 4°C. Biotinylated goat anti-rabbit 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 # SA1022) with DAB as the chromogen.

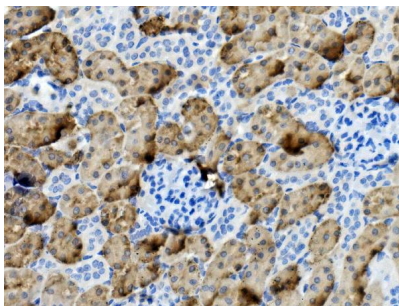


Figure 3. IHC analysis of PCK1/PEPC using anti-PCK1/PEPC antibody (A02022-3).

PCK1/PEPC was detected in paraffin-embedded section of rat kidney 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 1ug/ml rabbit anti-PCK1/PEPC Antibody (A02022-3) overnight at 4°C. Biotinylated goat anti-rabbit 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 # SA1022) with DAB as the chromogen.

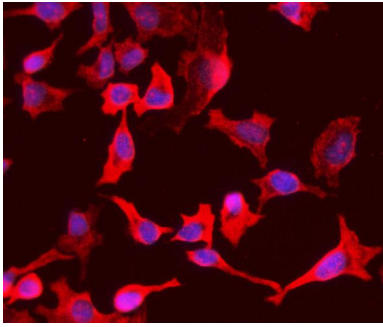


Figure 4. IF analysis of PCK1/PEPC using anti-PCK1/PEPC antibody (A02022-3). PCK1/PEPC was detected in immunocytochemical section of Hela cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 4ug/mL rabbit anti-PCK1/PEPC Antibody (A02022-3) overnight at 4°C. DyLight®594 Conjugated Goat Anti-Rabbit IgG (BA1142) 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.

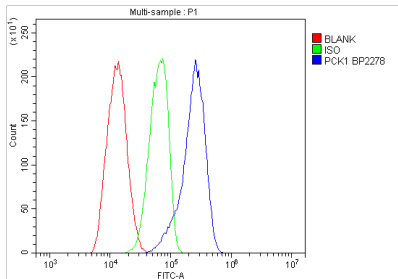


Figure 5. Flow Cytometry analysis of HL-60 cells using anti-PCK1/PEPC antibody (A02022-3). Overlay histogram showing HL-60 cells stained with A02022-3 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-PCK1/PEPC Antibody (A02022-3, 1ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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