

Anti-PKM2 Antibody Picoband™ (monoclonal, 11I4C3)

Catalog Number: M01173-1

About PKM

PKM (Pyruvate Kinase, Muscle), also known as PK3 or PKM2, is an enzyme that in humans is encoded by the PKM gene. The activity of pyruvate kinase subtype M2 is increased by fructose 1, 6-bisphosphate (Fru-1, 6-P2). By in situ hybridization, Popescu and Cheng (1990) mapped the THBP1 gene to 15q24-q25. Ashizawa et al. (1991) manipulated the intracellular Fru-1, 6-P2 concentration in several mammalian cell lines, including human, by varying the glucose concentration in the media. Using a novel proteomic screen for phosphotyrosine-binding proteins, Christofk et al. (2008) observed that PKM2 binds directly and selectively to tyrosine-phosphorylated peptides.

Overview

Product Name	Anti-PKM2 Antibody Picoband™ (monoclonal, 11I4C3)
Reactive Species	Human
Description	Boster Bio Anti-PKM2 Antibody Picoband™ (monoclonal, 11I4C3) catalog # M01173-1. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Monoclonal 11I4C3
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 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	Mouse
Uniprot ID	P14618

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence at the N-terminus of human PKM2, different from the related mouse sequence by five amino acids, and from the related rat sequence by four amino acids.
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Mouse IgG (EK1001) for Western blot, and HRP Conjugated anti-Mouse IgG Super Vision Assay Kit (SV0001-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Mouse IgG2b
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, 0.25-0.5 ug/ml, Human Immunohistochemistry(Paraffin-embedded Section), 2-5 ug/ml, Human Immunocytochemistry/Immunofluorescence, 5 ug/ml, Human Flow Cytometry, 1-3 ug/1x10⁶ cells, Human

Anti-PKM2 Antibody Picoband™ (monoclonal, 11I4C3) (M01173-1) Images

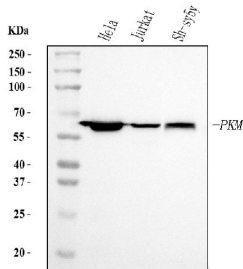


Figure 1. Western blot analysis of PKM2 using anti-PKM2 antibody (M01173-1).

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 HeLa whole cell lysates,
Lane 2: human Jurkat whole cell lysates,
Lane 3: human SH-SY5Y whole cell 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 mouse anti-PKM2 antigen affinity purified monoclonal antibody (Catalog # M01173-1) 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-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 PKM2 at approximately 60 kDa. The expected band size for PKM2 is at 60 kDa.

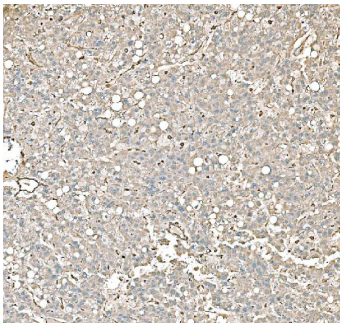


Figure 2. IHC analysis of PKM2 using anti-PKM2 antibody (M01173-1).

PKM2 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 mouse anti-PKM2 Antibody (M01173-1) 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.

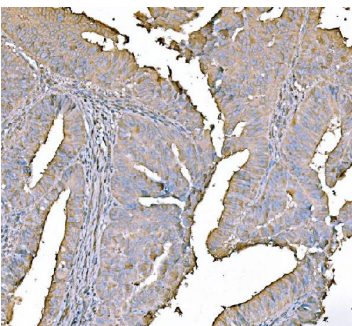
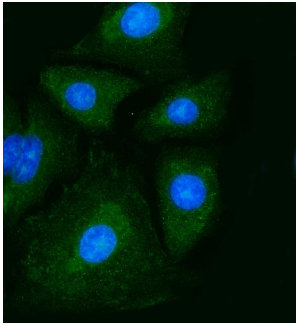


Figure 3. IHC analysis of PKM2 using anti-PKM2 antibody (M01173-1).

PKM2 was detected in a paraffin-embedded section of differentiated adenocarcinoma of the human rectum 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 mouse anti-PKM2 Antibody (M01173-1) 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. IF analysis of PKM2 using anti-PKM2 antibody (M01173-1).



PKM2 was detected in an immunocytochemical section of A549 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 mouse anti-PKM2 Antibody (M01173-1) 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.

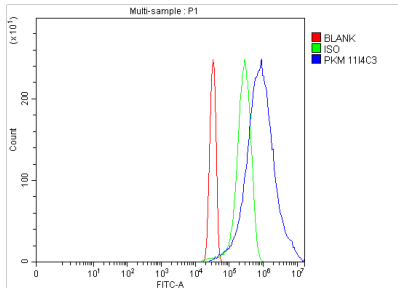


Figure 5. Flow Cytometry analysis of HeLa cells using anti-PKM2 antibody (M01173-1). Overlay histogram showing HeLa cells stained with M01173-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-PKM2 Antibody (M01173-1, 1 ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 ug/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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