

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

Catalog # ABO15123

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P14618
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-PKM2 Antibody Picoband™ (monoclonal, 11I4C3) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
Reconstitution	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.

Additional Information

Gene ID	5315
Other Names	Pyruvate kinase PKM, 2.7.1.40, Cytosolic thyroid hormone-binding protein, CTHBP, Opa-interacting protein 3, OIP-3, Pyruvate kinase 2/3, Pyruvate kinase muscle isozyme, Threonine-protein kinase PKM2, 2.7.11.1, Thyroid hormone-binding protein 1, THBP1, Tumor M2-PK, Tyrosine-protein kinase PKM2, 2.7.10.2, p58, PKM, OIP3 {ECO:0000303 PubMed:9466265}, PK2, PK3, PKM2
Calculated MW	57937
Application Details	Western blot, 0.25-0.5 µg/ml, Human Immunohistochemistry(Paraffin-embedded Section), 2-5 µg/ml, Human Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human Flow Cytometry, 1-3 µg/1x10 ⁶ cells, Human
Source	Eukaryota
Contents	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na ₂ HPO ₄ .
Clone Names	Clone: 11I4C3
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.
Purification	Immunogen affinity purified.
Storage	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.

Protein Information

Name	PKM
Synonyms	OIP3 {ECO:0000303 PubMed:9466265}, PK2,
Function	Catalyzes the final rate-limiting step of glycolysis by mediating the transfer of a phosphoryl group from phosphoenolpyruvate (PEP) to ADP, generating ATP (PubMed: 15996096 , PubMed: 1854723 , PubMed: 20847263). The ratio between the highly active tetrameric form and nearly inactive dimeric form determines whether glucose carbons are channeled to biosynthetic processes or used for glycolytic ATP production (PubMed: 15996096 , PubMed: 1854723 , PubMed: 20847263). The transition between the 2 forms contributes to the control of glycolysis and is important for tumor cell proliferation and survival (PubMed: 15996096 , PubMed: 1854723 , PubMed: 20847263).
Cellular Location	[Isoform M2]: Cytoplasm. Nucleus Note=Translocates to the nucleus in response to various signals, such as EGF receptor activation or apoptotic stimuli (PubMed:17308100, PubMed:22056988, PubMed:24120661). Nuclear translocation is promoted by acetylation by EP300 (PubMed:24120661). Deacetylation by SIRT6 promotes its nuclear export in a process dependent of XPO4, thereby suppressing its ability to activate transcription and promote tumorigenesis (PubMed:26787900).
Tissue Location	[Isoform M2]: Specifically expressed in proliferating cells, such as embryonic stem cells, embryonic carcinoma cells, as well as cancer cells.

Background

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.

Images

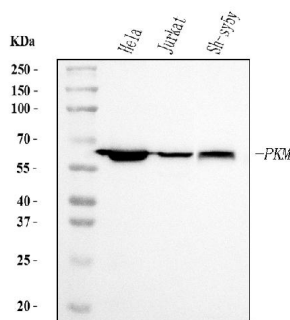


Figure 1. Western blot analysis of PKM2 using anti-PKM2 antibody (M01173-1).

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 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 PKM2 at approximately 60 kDa. The expected band size for PKM2 is at 60 kDa.

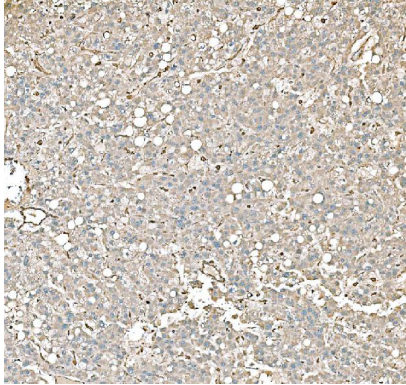


Figure 2. IHC analysis of PKM2 using anti-PKM2 antibody (M01173-1).

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 µg/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.

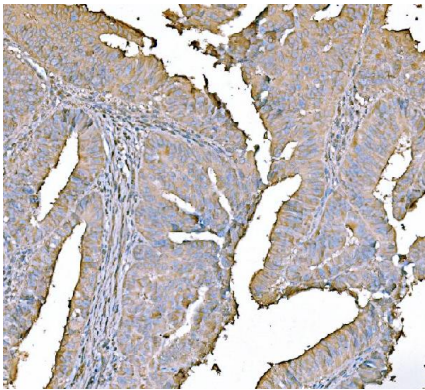


Figure 3. IHC analysis of PKM2 using anti-PKM2 antibody (M01173-1).

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 µg/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.

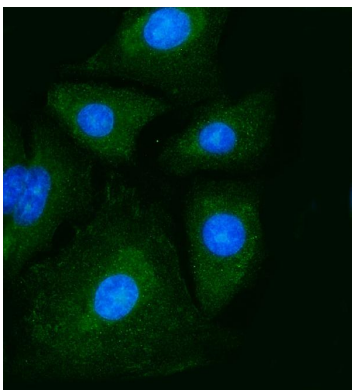
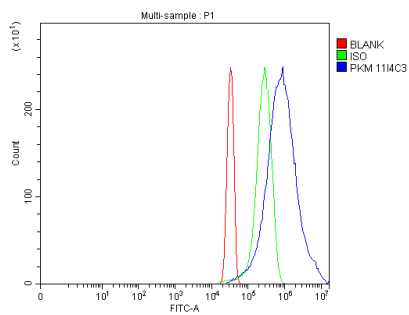


Figure 4. IF analysis of PKM2 using anti-PKM2 antibody (M01173-1).

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 µg/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 µg/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse



IgG (BA1126, 5-10 $\mu\text{g}/1 \times 10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 $\mu\text{g}/1 \times 10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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