Anti-PKM2 Antibody

R1603-5

Product Type:	Rabbit polyclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 58 kDa

Description: Glycolytic enzyme that catalyzes the transfer of a phosphoryl group from phosphoenolpyruvate (PEP) to ADP, generating ATP. Stimulates POU5F1-mediated transcriptional activation. Plays a general role in caspase independent cell death of tumor cells. 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. The transition between the 2 forms contributes to the control of glycolysis and is important for tumor cell proliferation and survival . Promotes in a STAT1-dependent manner, the expression of the immune checkpoint protein CD274 in ARNTL/BMAL1-deficient macrophages (By similarity).

Immunogen: Recombinant protein corresponding to C terminal of Human PKM2 .

Positive control: MDA-MB-231 cell lysate, SiHa cell lysate, NIH/3T3 cell lysate, rat heart tissue lysate, rat skeletal muscle tissue lysate, A549, rat brain tissue, human tonsil tissue, human colon cancer tissue, mouse testis tissue.

Subcellular location: Cytoplasm, Nucleus

Database links: SwissProt: P14618 Human | P52480 Mouse | P11980 Rat

Recommended Dilutions:

WB	1:1,000-1:5,000
IF-Cell	1:50-1:200
IHC-P	1:50-1:200
FC	1:50-1:100

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

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Immunogen affinity purified.

Images

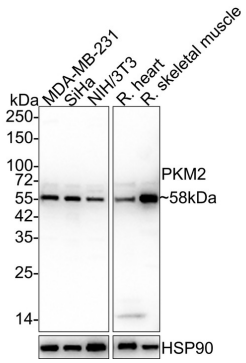

Fig1: Western blot analysis of PKM2 on different lysates with Rabbit anti-PKM2 antibody (R1603-5) at 1/1,000 dilution.

Lane 1: MDA-MB-231 cell lysate (15 µg/Lane)
Lane 2: SiHa cell lysate (15 µg/Lane)
Lane 3: NIH/3T3 cell lysate (15 µg/Lane)
Lane 4: Rat heart tissue lysate (30 µg/Lane)
Lane 5: Rat skeletal muscle tissue lysate (30 µg/Lane)

Predicted band size: 58 kDa
Observed band size: 58 kDa

Exposure time: 4 seconds;

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 (R1603-5) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

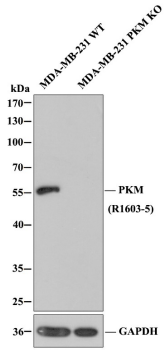

Fig2: All lanes: Western blot analysis of PKM with anti-PKM antibody (R1603-5) at 1:500 dilution.

Lane 1: Wild-type MDA-MB-231 whole cell lysate.
Lane 2: PKM knockout MDA-MB-231 whole cell lysate.

R1603-5 was shown to specifically react with PKM in wild-type MDA-MB-231 cells. No band was observed when PKM knockout sample was tested. Wild-type and PKM knockout samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (R1603-5, 1/500) and Loading control antibody (Rabbit anti-GAPDH , ET1601-4, 1/10,000) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG-HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

Cell lysate was provided by Ubigene Biosciences (Ubigene Biosciences Co., Ltd., Guangzhou, China).

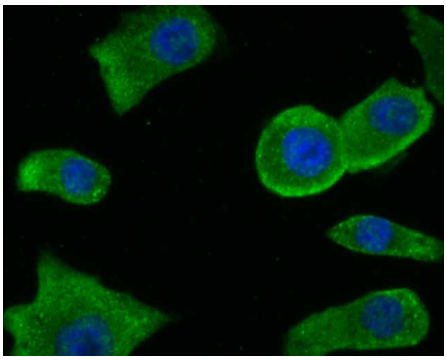

Fig3: ICC staining of PKM2 in A549 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (R1603-5, 1/100) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/100 dilution. The nuclear counter stain is DAPI (blue).

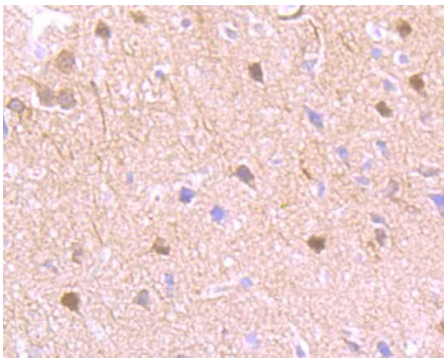

Fig4: Immunohistochemical analysis of paraffin-embedded rat brain tissue using anti-PKM2 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (R1603-5, 1/1200) for 30 minutes 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.

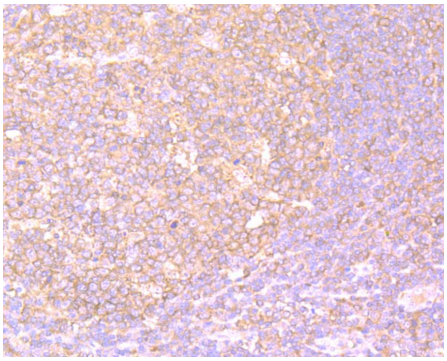

Fig5: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-PKM2 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (R1603-5, 1/1200) for 30 minutes 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.

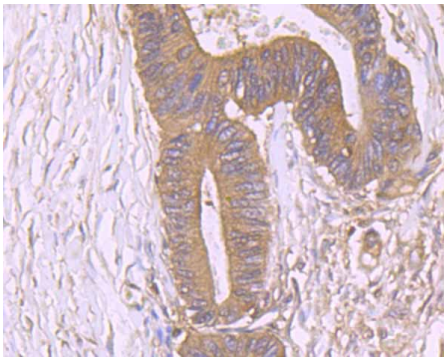

Fig6: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue using anti-PKM2 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (R1603-5, 1/200) for 30 minutes 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.

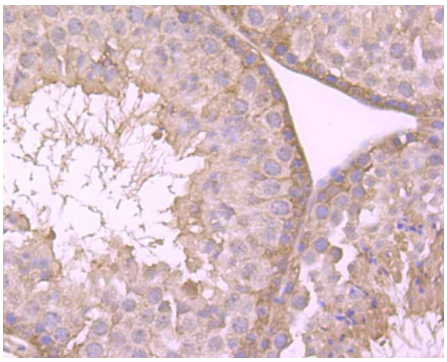

Fig7: Immunohistochemical analysis of paraffin-embedded mouse testis tissue using anti-PKM2 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (R1603-5, 1/200) for 30 minutes 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.

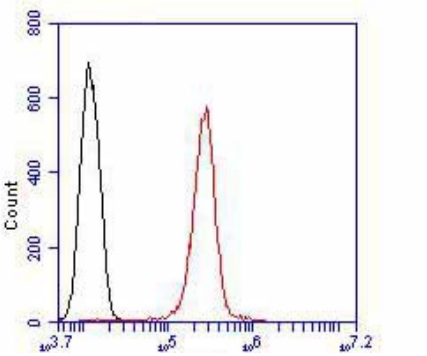

Fig8: Flow cytometric analysis of PKM2 was done on A549 cells. The cells were fixed, permeabilized and stained with the primary antibody (R1603-5, 1/100) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated goat anti-rabbit IgG Secondary antibody at 1/500 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Stetak A.et.al.Nuclear translocation of the tumor marker pyruvate kinase M2 induces programmed cell death.Cancer Res. 67:1602-1608(2007).