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).
lmmunogen:	Synthetic peptide within human PKM2 aa 381-430.
Positive control:	HeLa cell lysate, MCF7 cell lysate, A549 cell lysate, NIH/3T3 cell lysate, C6 cell lysate, HeLa, NIH/3T3, C6, human liver tissue, mouse liver tissue.
Subcellular location:	Cytoplasm, Nucleus
Database links:	SwissProt: P14618 Human P52480 Mouse P11980 Rat
Recommended Dilutions: WB IF-Cell IHC-P FC	1:2,000-1:5,000 1:200 1:10,000 1:1,000
Storage Buffer:	PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Shipped at 4 $^\circ\!\mathrm{C}$. Store at +4 $^\circ\!\mathrm{C}$ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20 $^\circ\!\mathrm{C}$ long term.
Purity:	Immunogen affinity purified.

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Images



Fig1: Western blot analysis of PKM2 on different lysates with Rabbit anti-PKM2 antibody (R1603-5) at 1/2,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane) Lane 2: MCF7 cell lysate (20 µg/Lane) Lane 3: A549 cell lysate (20 µg/Lane) Lane 4: NIH/3T3 cell lysate (20 µg/Lane) Lane 5: C6 cell lysate (20 µg/Lane) Lane 6: Mouse skeletal muscle tissue lysate (negative) (40 µg/Lane)

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

Exposure time: 13 seconds; ECL: K1801;

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 1/2,000 dilution was used in 5% NFDM/TBST at 4° C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig2: Immunocytochemistry analysis of HeLa cells labeling PKM2 with Rabbit anti-PKM2 antibody (R1603-5) at 1/200 dilution.



Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-PKM2 antibody (R1603-5) at 1/200 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at $+4^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor 1594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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Fig3: Immunocytochemistry analysis of NIH/3T3 cells labeling PKM2 with Rabbit anti-PKM2 antibody (R1603-5) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-PKM2 antibody (R1603-5) at 1/200 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at $+4^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor 1594, HA1126) was used as the secondary antibody at 1/1,000 dilution.



Fig4: Immunocytochemistry analysis of C6 cells labeling PKM2 with Rabbit anti-PKM2 antibody (R1603-5) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-PKM2 antibody (R1603-5) at 1/200 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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Fig5: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-PKM2 antibody (R1603-5) at 1/10,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (R1603-5) at 1/10,000 dilution for 1 hour 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 mouse liver tissue with Rabbit anti-PKM2 antibody (R1603-5) at 1/10,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (R1603-5) at 1/10,000 dilution for 1 hour 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.



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Fig7: Flow cytometric analysis of HeLa cells labeling PKM2.

Cells were fixed and permeabilized. Then stained with the primary antibody (R1603-5, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4 °C for an hour, the cells were stained with a iFluor TM 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4 °C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Fig8: Flow cytometric analysis of NIH/3T3 cells labeling PKM2.

Cells were fixed and permeabilized. Then stained with the primary antibody (R1603-5, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C for an hour, the cells were stained with a iFluor TM 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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Fig9: Flow cytometric analysis of C6 cells labeling PKM2.

Cells were fixed and permeabilized. Then stained with the primary antibody (R1603-5, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C for an hour, the cells were stained with a iFluorTM 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4°C. 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. Wang J et al. Lactylation of PKM2 Suppresses Inflammatory Metabolic Adaptation in Pro-inflammatory Macrophages. Int J Biol Sci. 2022 Oct
- 2. Wu Y et al. Phosphoglycerate dehydrogenase activates PKM2 to phosphorylate histone H3T11 and attenuate cellular senescence. Nat Commun. 2023 Mar

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

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