Anti-Hexokinase II Antibody [PSH07-97]

HA722933

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Monkey
Applications:	WB, IHC-P, IF-Cell, FC, IP
Molecular Wt:	Predicted band size: 102 kDa
Clone number:	PSH07-97

Description: Hexokinase 2 also known as HK2 is an enzyme which in humans is encoded by the HK2 gene on chromosome 2. Hexokinases phosphorylate glucose to produce glucose-6-phosphate (G6P), the first step in most glucose metabolism pathways. This gene encodes hexokinase 2, the predominant form found in skeletal muscle. It localizes to the outer membrane of mitochondria. As an isoform of hexokinase and a member of the sugar kinase family, HK2 catalyzes the rate-limiting and first obligatory step of glucose metabolism, which is the ATP-dependent phosphorylation of glucose to G6P. Physiological levels of G6P can regulate this process by inhibiting HK2 as negative feedback, though inorganic phosphate (Pi) can relieve G6P inhibition. Pi can also directly regulate HK2, and the double regulation may better suit its anabolic functions. By phosphorylating glucose, HK2 effectively prevents glucose from leaving the cell and, thus, commits glucose to energy metabolism. Moreover, its localization and attachment to the OMM promotes the coupling of glycolysis to mitochondrial oxidative phosphorylation, which greatly enhances ATP production to meet the cell's energy demands. Specifically, HK2 binds VDAC to trigger opening of the channel and release mitochondrial ATP to further fuel the glycolytic process.

Immunogen: Recombinant protein within

Positive control: HeLa cell lysate, HCT 116 cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, COS-1 cell lysate, Mouse skeletal muscle tissue lysate, Mouse testis tissue lysate, Rat skeletal muscle tissue lysate, Rat testis tissue lysate, NIH/3T3, PC-12, human skeletal muscle tissue, mouse skeletal muscle tissue, rat skeletal muscle tissue.

Subcellular location: Mitochondrion outer membrane, Cytoplasm, cytosol.

Database links: SwissProt: P52789 Human | O08528 Mouse | P27881 Rat

Recommended Dilutions:

WB	1:1,000
IHC-P	1:50
IF-Cell	1:50-1:100
FC	1:1,000
IP	1-2μg/sample

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

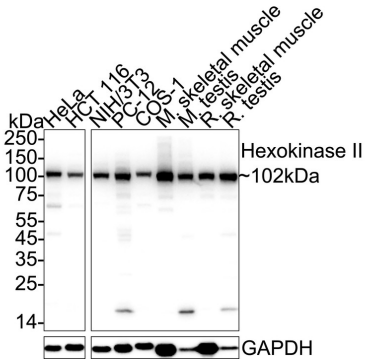

Fig1: Western blot analysis of Hexokinase II on different lysates with Rabbit anti-Hexokinase II antibody (HA722933) at 1/1,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: HCT 116 cell lysate (20 µg/Lane)
Lane 3: NIH/3T3 cell lysate (20 µg/Lane)
Lane 4: PC-12 cell lysate (20 µg/Lane)
Lane 5: COS-1 cell lysate (20 µg/Lane)
Lane 6: Mouse skeletal muscle tissue lysate (40 µg/Lane)
Lane 7: Mouse testis tissue lysate (40 µg/Lane)
Lane 8: Rat skeletal muscle tissue lysate (40 µg/Lane)
Lane 9: Rat testis tissue lysate (40 µg/Lane)

Predicted band size: 102 kDa
Observed band size: 102 kDa

Exposure time: 8 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 (HA722933) 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.

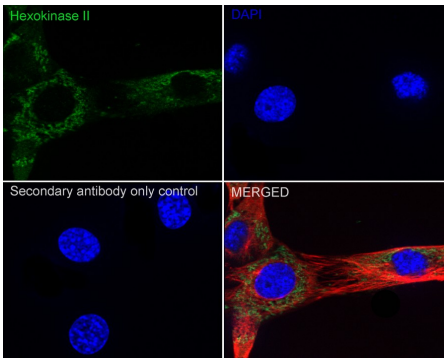

Fig2: Immunocytochemistry analysis of NIH/3T3 cells labeling Hexokinase II with Rabbit anti-Hexokinase II antibody (HA722933) at 1/50 dilution.

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-Hexokinase II antibody (HA722933) at 1/50 dilution in 1% BSA in PBST overnight at 4 ℃. 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℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

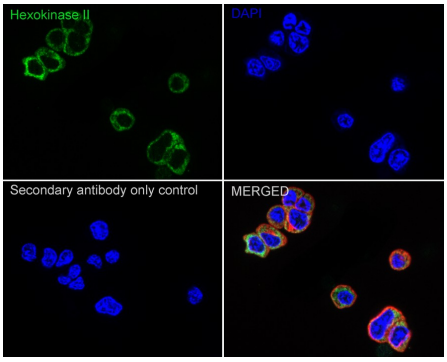

Fig3: Immunocytochemistry analysis of PC-12 cells labeling Hexokinase II with Rabbit anti-Hexokinase II antibody (HA722933) at 1/100 dilution.

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-Hexokinase II antibody (HA722933) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. 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℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

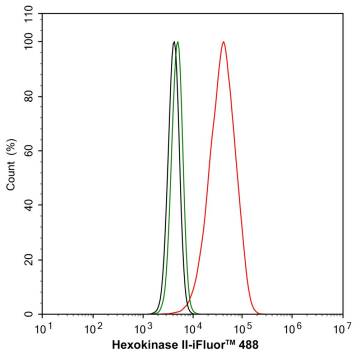

Fig4: Flow cytometric analysis of NIH/3T3 cells labeling Hexokinase II.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722933, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

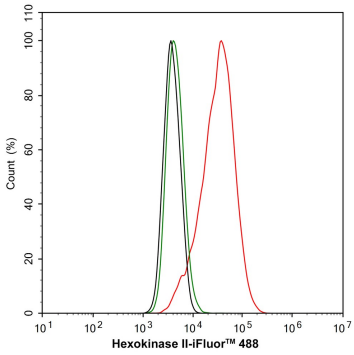

Fig5: Flow cytometric analysis of PC-12 cells labeling Hexokinase II.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722933, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

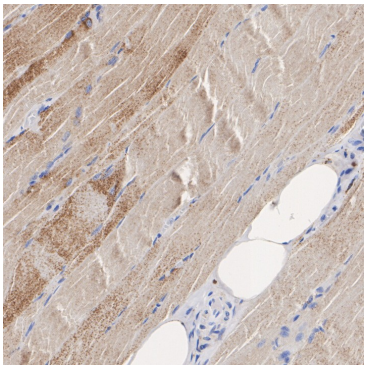

Fig6: Immunohistochemical analysis of paraffin-embedded human skeletal muscle tissue with Rabbit anti-Hexokinase II antibody (HA722933) at 1/50 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (HA722933) at 1/50 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.

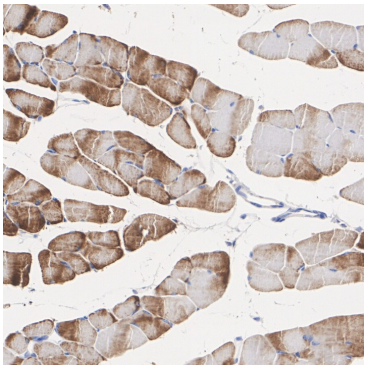

Fig7: Immunohistochemical analysis of paraffin-embedded mouse skeletal muscle tissue with Rabbit anti-Hexokinase II antibody (HA722933) at 1/50 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (HA722933) at 1/50 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.

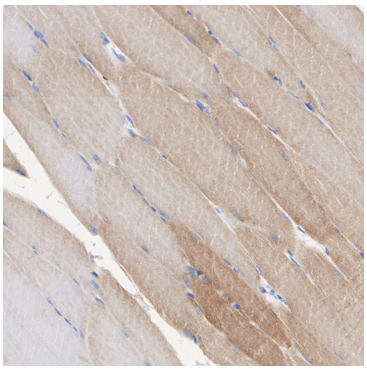

Fig8: Immunohistochemical analysis of paraffin-embedded rat skeletal muscle tissue with Rabbit anti-Hexokinase II antibody (HA722933) at 1/50 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (HA722933) at 1/50 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.

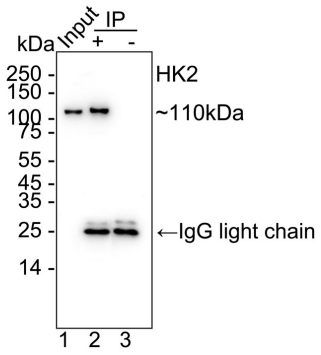

Fig9: Hexokinase II was immunoprecipitated from 0.2 mg HeLa cell lysate with HA722933 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA722933 at 1/2,000 dilution. Mouse Anti-Rabbit IgG kappa light chain secondary antibody (M1208-2) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: HA722933 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of HA722933 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 6 seconds; ECL: K1801

Background References

1. Rho H et al. Hexokinase 2-mediated gene expression via histone lactylation is required for hepatic stellate cell activation and liver fibrosis. Cell Metab. 2023 Aug
2. Leng L et al. Microglial hexokinase 2 deficiency increases ATP generation through lipid metabolism leading to beta-amyloid clearance. Nat Metab. 2022 Oct