

Anti-PFKM Antibody [JU53-31]

ET7106-97



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC
Molecular Wt:	Predicted band size: 85 kDa
Clone number:	JU53-31

Description: Catalyzes the phosphorylation of D-fructose 6-phosphate to fructose 1,6-bisphosphate by ATP, the first committing step of glycolysis. In human PFK exists as a system of 3 types of subunits, PFKM (muscle), PFKL (liver) and PFKP (platelet) isoenzymes.

Immunogen: Synthetic peptide within Human PFKM aa 107-156 / 780.

Positive control: HeLa cell lysate, Ramos cell lysate, mouse heart tissue lysate, mouse skeletal muscle tissue lysate, rat heart tissue lysate, rat skeletal muscle tissue lysate, A549 cell lysate, LOVO, PC-3M, SH-SY5Y, rat brain tissue, human liver carcinoma tissue, mouse skeletal muscle tissue, human thyroid tissue, human kidney tissue.

Subcellular location: Cytoplasm.

Database links: SwissProt: P08237 Human | P47857 Mouse | P47858 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IF-Cell	1:50-1:200
IHC-P	1:50-1:200
FC	1:50-1:100

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

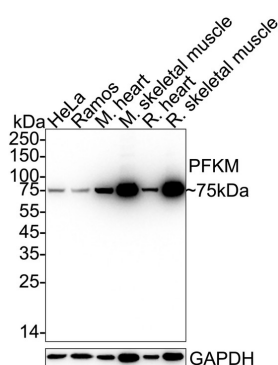
Technical:0086-571-89986345

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Images

Fig1: Western blot analysis of PFKM on different lysates with Rabbit anti-PFKM antibody (ET7106-97) at 1/1,000 dilution.



Lane 1: HeLa cell lysate
 Lane 2: Ramos cell lysate
 Lane 3: Mouse heart tissue lysate
 Lane 4: Mouse skeletal muscle tissue lysate
 Lane 5: Rat heart tissue lysate
 Lane 6: Rat skeletal muscle tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 85 kDa

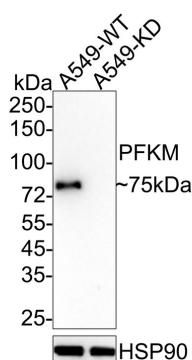
Observed band size: 75 kDa

Exposure time: 10 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDN/TBST for 1 hour at room temperature. The primary antibody (ET7106-97) at 1/1,000 dilution was used in 5% NFDN/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: Western blot analysis of PFKM on different lysates with Rabbit anti-PFKM antibody (ET7106-97) at 1/1,000 dilution.



Lane 1: A549-WT cell lysate
 Lane 2: A549-KD PFKM cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 85 kDa

Observed band size: 75 kDa

Exposure time: 3 minutes; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDN/TBST for 1 hour at room temperature. The primary antibody (ET7106-97) at 1/1,000 dilution was used in 5% NFDN/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.

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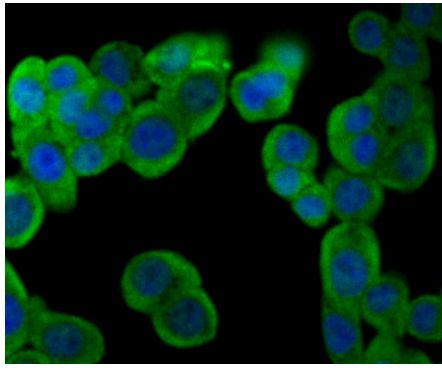


Fig3: ICC staining of PFKM in LOVO cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET7106-97, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

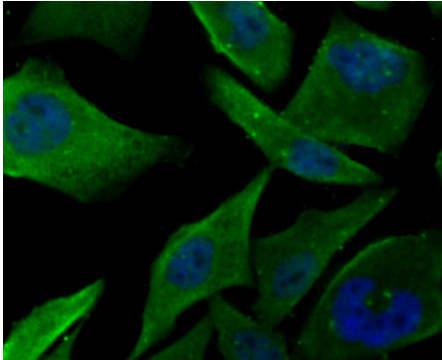


Fig4: ICC staining of PFKM in PC-3M cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET7106-97, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

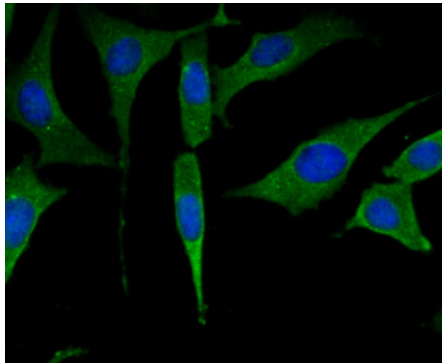


Fig5: ICC staining of PFKM in SH-SY5Y cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET7106-97, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

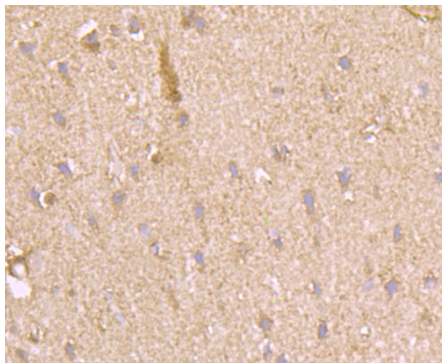


Fig6: Immunohistochemical analysis of paraffin-embedded rat brain tissue using anti-PFKM antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET7106-97, 1/50) 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.

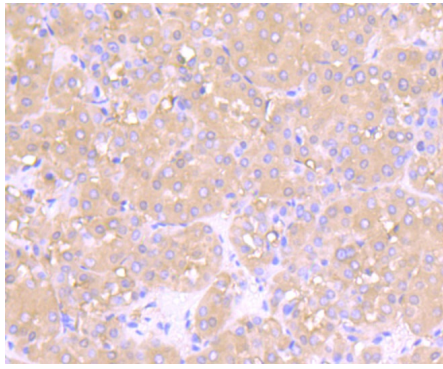


Fig7: Immunohistochemical analysis of paraffin-embedded human liver carcinoma tissue using anti-PFKM antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET7106-97, 1/50) 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.

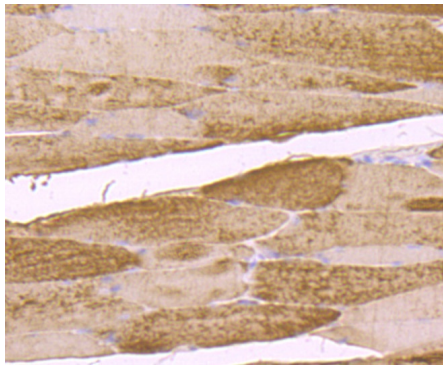


Fig8: Immunohistochemical analysis of paraffin-embedded mouse skeletal muscle tissue using anti-PFKM antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET7106-97, 1/50) 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.

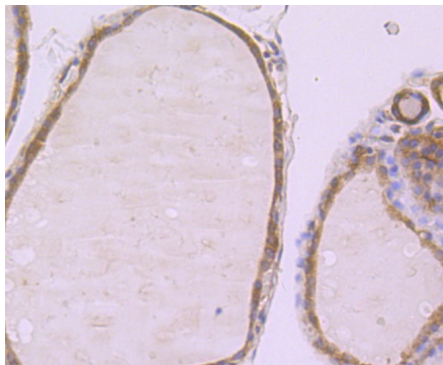


Fig9: Immunohistochemical analysis of paraffin-embedded human thyroid tissue using anti-PFKM antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET7106-97, 1/50) 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.

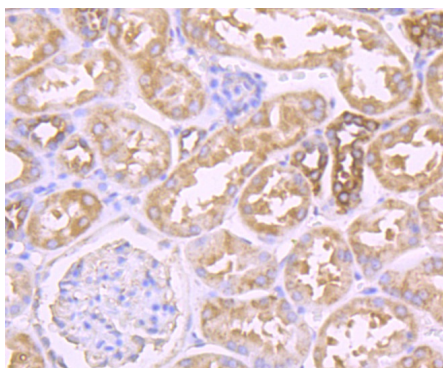


Fig10: Immunohistochemical analysis of paraffin-embedded human kidney tissue using anti-PFKM antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET7106-97, 1/50) 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.

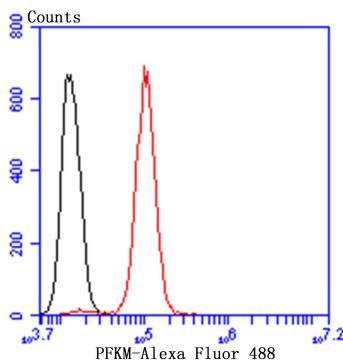


Fig11: Flow cytometric analysis of PFKM was done on SH-SY5Y cells. The cells were fixed, permeabilized and stained with the primary antibody (ET7106-97, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 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. Tsujino S et al. Identification of three novel mutations in non-Ashkenazi Italian patients with muscle phosphofructokinase deficiency. *Am J Hum Genet* 54:812-819 (1994).
2. Raben N et al. Functional expression of human mutant phosphofructokinase in yeast: genetic defects in French Canadian and Swiss patients with phosphofructokinase deficiency. *Am J Hum Genet* 56:131-141 (1995).

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