Anti-PFKFB3 Antibody [JM43-43]

ET1705-66

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC, IP, IF-Tissue
Molecular Wt:	Predicted band size: 60 kDa
Clone number:	JM43-43

Description: Among the enzymes playing role in glycolysis,four allosteric PFKFB enzymes 1–4 expressed by four independent PFKFB genes, catalyze the rate-limiting phosphorylation of fructose-6-phosphate to fructose-1, 6-bisphosphate, using ATP as the energy source in the glycolysis pathway. Among these four allosteric enzymes, PFKFB3 enzyme retains the highest Kinase/Biphosphatase activity ratio and is expressed by PFKFB3 gene which has been demonstrated to be highly expressed in leukemic cells and in solid tumors. Moreover, mitogenic, hypoxic and inflammatory conditions have an inductive effect on the expression of PFKFB3. Hence upregulation of PFKFB genes specific to cancer cells compared to their normal counterparts (from the same patients) with more robust over-expression in breast and lung cancer make it a more appropriate target.

Immunogen: Recombinant protein within Human PFKFB3 aa 260-520 / 520.

Positive control: HeLa cell lysate, PC-12 cell lysate, A431 cell lysate, A549 cell lysate, human kidney tissue, A549, Hela, rat kidney tissue, mouse colon tissue, A431.

Subcellular location: Cytosol. Nucleoplasm.

Database links: SwissProt: Q16875 Human | O35552 Rat | Q3U3S6 Mouse

Recommended Dilutions:

WB	1:500-1:1,000
IF-Cell	1:50-1:200
IHC-P	1:1,000
FC	1:50-1:100
IP	Use at an assay dependent concentration.
IF-Tissue	1:200

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

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

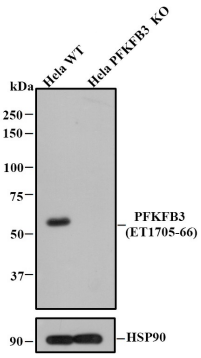

Fig1: All lanes: Western blot analysis of PFKFB3 with anti-PFKFB3 antibody [JM43-43] (ET1705-66) at 1/500 dilution.

Lane 1: Wild-type Hela whole cell lysate.
Lane 2: PFKFB3 knockout Hela whole cell lysate.

ET1705-66 was shown to specifically react with PFKFB3 in wild-type Hela cells. No band was observed when PFKFB3 knockout sample was tested. Wild-type and PFKFB3 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 Anti-PFKFB3 antibody (ET1705-66, 1/500) and Anti-HSP90 antibody (ET1605-56, 1/10,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG H&L (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).

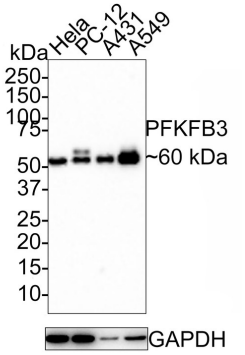

Fig2: Western blot analysis of PFKFB3 on different lysates with Rabbit anti-PFKFB3 antibody (ET1705-66) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: PC-12 cell lysate
Lane 3: A431 cell lysate
Lane 4: A549 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 60 kDa
Observed band size: 60 kDa

Exposure time: 2 minutes;

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 (ET1705-66) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

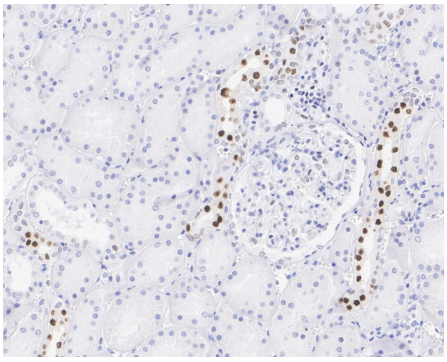

Fig3: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-PFKFB3 antibody (ET1705-66) at 1/1,000 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (ET1705-66) at 1/1,000 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.

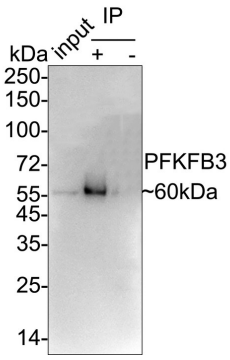

Fig4: PFKFB3 was immunoprecipitated from 0.2 mg HeLa cell lysate with ET1705-66 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using ET1705-66 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: ET1705-66 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of ET1705-66 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 2 minutes; ECL: K1802

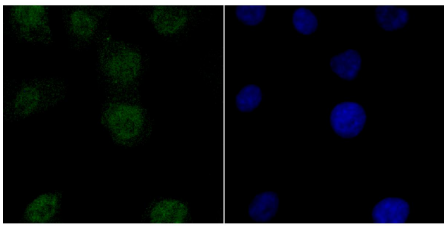

Fig5: ICC staining of PFKFB3 in A549 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1705-66, 1/50) for 1 hour at 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).

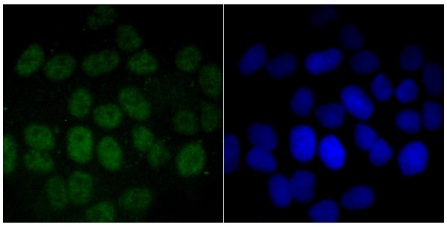

Fig6: ICC staining of PFKFB3 in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1705-66, 1/50) for 1 hour at 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).

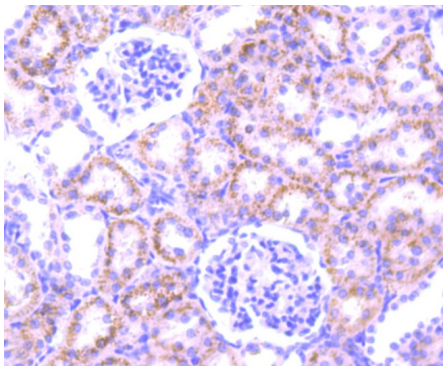

Fig7: Immunohistochemical analysis of paraffin-embedded rat kidney tissue using anti-PFKFB3 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1705-66, 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.

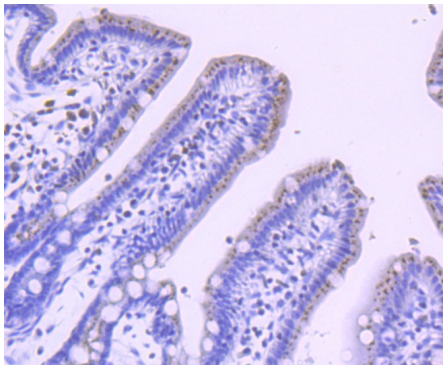

Fig8: Immunohistochemical analysis of paraffin-embedded mouse colon tissue using anti-PFKFB3 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1705-66, 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.

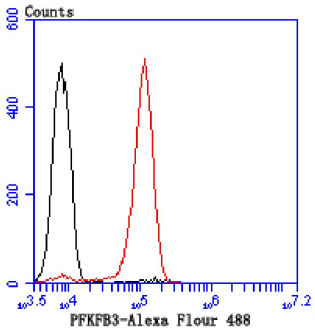

Fig9: Flow cytometric analysis of PFKFB3 was done on A431 cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1705-66, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit 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. Jiang H et al. PFKFB3-Driven Macrophage Glycolytic Metabolism Is a Crucial Component of Innate Antiviral Defense. J Immunol 197:2880-90 (2016).
2. Lu Q et al. Akt inhibition attenuates rasfonin-induced autophagy and apoptosis through the glycolytic pathway in renal cancer cells. Cell Death Dis 6:e2005 (2015).