Anti-PDK1 Antibody [JA67-30]

ET1704-66

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IP, FC, IF-Cell, IF-Tissue
Molecular Wt:	Predicted band size: 49 kDa
Clone number:	JA67-30

Description: Mitochondrial pyruvate dehydrogenase (PDH) catalyzes the oxidative decarboxylation of pyruvate and plays a central role in the regulation of homeostasis of carbohydrate fuels in mammals. PDH activity is controlled by a phosphorylation/dephosphorylation cycle, phosphorylation leading to inactivation and dephosphorylation leading to reactivation of PDH. The phosphorylation of PDH is catalyzed by pyruvate dehydrogenase kinase (PDK), the activity of which is stimulated by the products of PDH catalysis. PDK1 consists of alpha and beta subunits; the kinase activity resides in the alpha subunit. Three PDK isoenzymes have been identified in humans (PDK1, 2 and 3) and two have been identified in rodent (PDK1 and 2).

Immunogen: Synthetic peptide within Human PDK1 aa 16-65 / 436.

Positive control: Mouse heart tissue lysate, rat heart tissue lysate, Hela, NIH/3T3, mouse heart tissue, rat heart tissue.

Subcellular location: Mitochondrion matrix.

Database links: SwissProt: Q15118 Human | Q8BFP9 Mouse | Q63065 Rat

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:50-1:200
IHC-P	1:2,000
FC	1:50-1:100
IF-Tissue	1:200-1:400

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

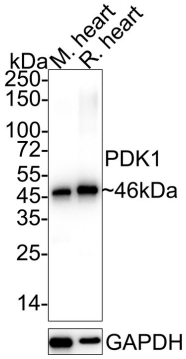

Fig1: Western blot analysis of PDK1 on different lysates with Rabbit anti-PDK1 antibody (ET1704-66) at 1/5,000 dilution.

Lane 1: Mouse heart tissue lysate
Lane 2: Rat heart tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 49 kDa
Observed band size: 46 kDa

Exposure time: 10 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 (ET1704-66) at 1/5,000 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.

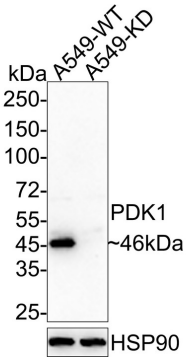

Fig2: Western blot analysis of PDK1 on different lysates with Rabbit anti-PDK1 antibody (ET1704-66) at 1/1,000 dilution.

Lane 1: A549-WT cell lysate
Lane 2: A549-KD PDK1 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 49 kDa
Observed band size: 46 kDa

Exposure time: 3 minutes; 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 (ET1704-66) at 1/1,000 dilution was used in primary antibody diluent at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

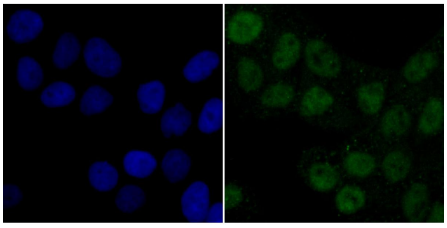

Fig3: ICC staining of PDK1 in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1704-66, 1/50) for 1 hour at room 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).

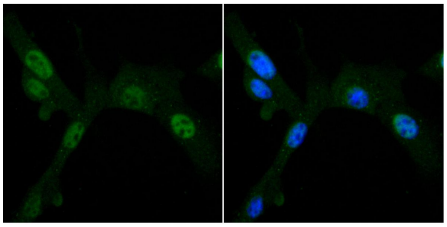

Fig4: ICC staining of PDK1 in NIH/3T3 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1704-66, 1/50) for 1 hour at 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).

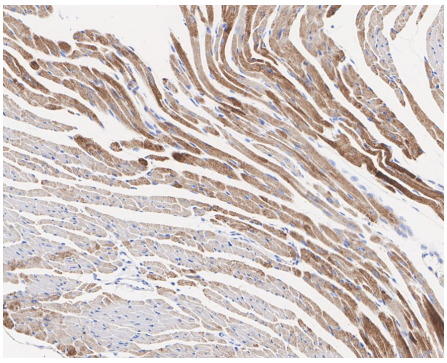

Fig5: Immunohistochemical analysis of paraffin-embedded mouse heart tissue with Rabbit anti-PDK1 antibody (ET1704-66) at 1/2,000 dilution.

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 (ET1704-66) at 1/2,000 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.

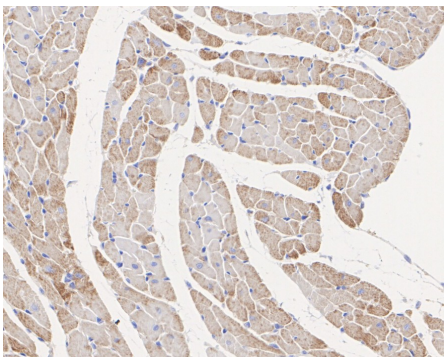

Fig6: Immunohistochemical analysis of paraffin-embedded rat heart tissue with Rabbit anti-PDK1 antibody (ET1704-66) at 1/2,000 dilution.

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 (ET1704-66) at 1/2,000 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.

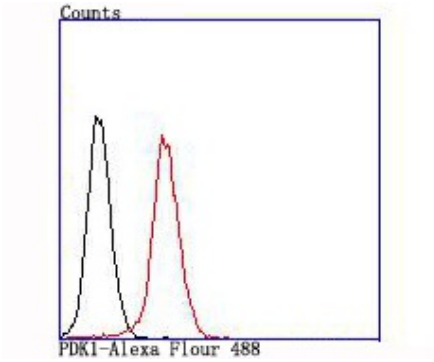

Fig7: Flow cytometric analysis of PDK1 was done on NIH/3T3 cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1704-66, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat 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).

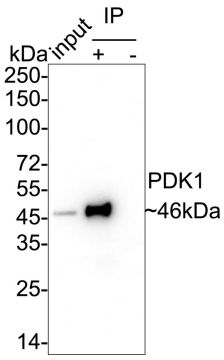

Fig8: PDK1 was immunoprecipitated from 0.2 mg mouse heart tissue lysate with ET1704-66 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using ET1704-66 at 1/2,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: Mouse heart tissue lysate (input)
Lane 2: ET1704-66 IP in mouse heart tissue lysate
Lane 3: Rabbit IgG instead of ET1704-66 in mouse heart tissue lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 3 minutes; ECL: K1801

Background References

1. Du L et al. Overexpression of PIK3CA in murine head and neck epithelium drives tumor invasion and metastasis through PDK1 and enhanced TGF signaling. Oncogene 35:4641-52 (2016).
2. Lelliott CJ et al. Monoclonal antibody targeting of fibroblast growth factor receptor 1c ameliorates obesity and glucose intolerance via central mechanisms. PLoS One 9:e112109 (2014).