Anti-LDHB Antibody

0807-1

Product Type:	Rabbit polyclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, FC, IHC-P
Molecular Wt:	Predicted band size: 37 kDa

Description: Lactate dehydrogenase (LDH) is an enzyme present in a wide variety of organisms, including plants and animals. It catalyses the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD+. In medicine, LDH is often used as a marker of tissue breakdown as LDH is abundant in red blood cells and can function as a marker for hemolysis. In mammals, three types of LDH subunits (35 kDa) are encoded by the genes Ldh-A, Ldh-B, and Ldh-C. Lactate dehydrogenase B (LDH-B, heart subunit, LDH-H) is involved in the conversion of L-lactate and NAD to pryruvate and NADH and it is predominantly localized in the heart tissue. Similar to other LDH subunit, LDH-B is considered to be an important marker for germ cell tumor.

Immunogen: Synthetic peptide within Human LDH-B2 aa 285-334 / 334.

Positive control: Hela cell lysate, Jurkat cell lysate, Daudi cell lysate, HL-60 cell lysate, A549, rat kidney tissue, human kidney tissue, mouse kidney tissue.

Subcellular location: Cytoplasm, Membrane, Mitochondrion, Mitochondrion inner membrane.

Database links: SwissProt: P07195 Human | P16125 Mouse | P42123 Rat

Recommended Dilutions:

WB	1:500-1:1,000
IF-Cell	1:200
FC	1:500-1:1,000
IHC-P	1:200-1:500

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 25% 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: Immunogen affinity purified.

Images

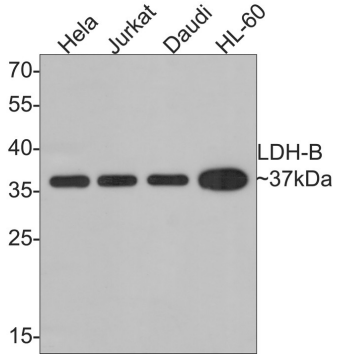

Fig1: Western blot analysis of LDHB on different lysates with Rabbit anti-LDHB antibody (0807-1) at 1/500 dilution.

Lane 1: Hela cell lysate
Lane 2: Jurkat cell lysate
Lane 3: Daudi cell lysate
Lane 4: HL-60 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 37 kDa
Observed band size: 37 kDa

Exposure time: 2 minutes;

12% 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 (0807-1) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

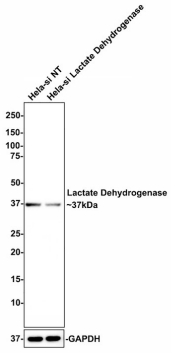

Fig2: Western blot analysis of Lactate Dehydrogenase on different lysates with Rabbit anti-Lactate Dehydrogenase antibody (0807-1) at 1/1,000 dilution.

Lane 1: Hela-si NT cell lysate
Lane 2: Hela-si Lactate Dehydrogenase cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 37 kDa
Observed band size: 37 kDa

Exposure time: 3 minutes;

4-20% SDS-PAGE gel.

0807-1 was shown to specifically react with Lactate Dehydrogenase in Hela-si NT cells. Weakened band was observed when Hela-si Lactate Dehydrogenase sample was tested. Hela-si NT and Hela-si Lactate Dehydrogenase 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 antibody (0807-1, 1/1,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% BSA at room temperature for 2 hours.

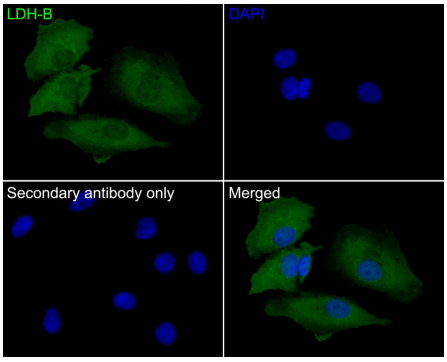

Fig3: Immunocytochemistry analysis of A549 cells labeling LDHB with Rabbit anti-LDHB antibody (0807-1) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-LDHB antibody (0807-1) at 1/200 dilution in 2% negative goat serum 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.

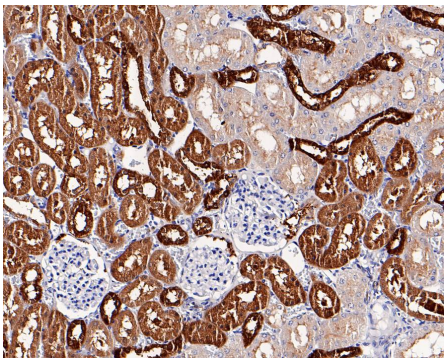

Fig4: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-LDHB antibody (0807-1) at 1/200 dilution.

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 (0807-1) at 1/200 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.

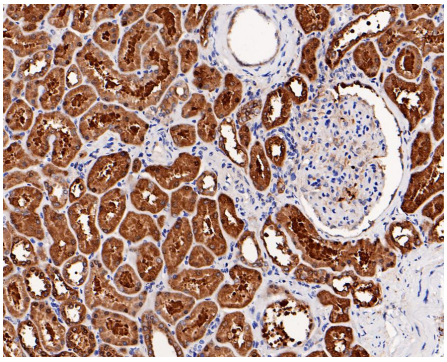

Fig5: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-LDHB antibody (0807-1) at 1/200 dilution.

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 (0807-1) at 1/200 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.

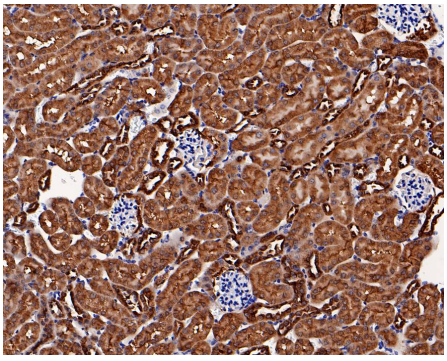

Fig6: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-LDHB antibody (0807-1) at 1/200 dilution.

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 (0807-1) at 1/200 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

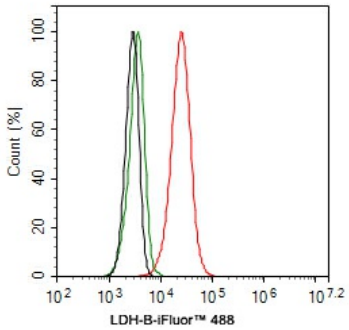

Fig7: Flow cytometric analysis of A549 cells labeling LDHB.

Cells were fixed and permeabilized. Then stained with the primary antibody (0807-1, 1ug/ml) (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).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Takeno T., Li S.S.-L.; "Structure of the human lactate dehydrogenase B gene."; Biochem. J. 257:921-924(1989).
2. The MGC Project Team; "The status, quality, and expansion of the NIH full-length cDNA project: the Mammalian Gene Collection (MGC)."; Genome Res. 14:2121-2127(2004).
3. Sakai I., Sharief F.S., Pan Y.-C.E., Li S.S.-L.; "The cDNA and protein sequences of human lactate dehydrogenase B."; Biochem. J. 248:933-936(1987).