# Anti-LDHA Antibody

## ER00702

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|---|---|
| **Product Type:** | Rabbit polyclonal IgG, primary antibodies |
| **Species reactivity:** | Human, Mouse, Rat |
| **Applications:** | WB, IHC-P, FC, IF-Cell |
| **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 LDHA aa 1-50 / 332.

**Positive control:** A431 cell lysate, MCF7 cell lysate, NIH/3T3 cell lysate, RAW264.7 cell lysate, C6 cell lysate, PC-12 cell lysate, human liver tissue, mouse liver tissue, rat liver tissue, A431, RAW264.7, C6.

**Subcellular location:** Cytoplasm.

**Database links:** SwissProt: P00338 Human | P06151 Mouse | P04642 Rat

**Recommended Dilutions:**

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| **WB** | 1:5,000 |
| **IHC-P** | 1:1,000 |
| **FC** | 1:1,000 |
| **IF-Cell** | 1:100 |

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

## Images

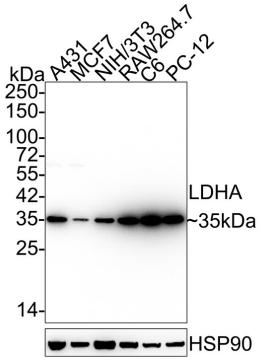


**Fig1:** Western blot analysis of LDHA on different lysates with Rabbit anti-LDHA antibody (ER00702) at 1/5,000 dilution.

Lane 1: A431 cell lysate
Lane 2: MCF7 cell lysate
Lane 3: NIH/3T3 cell lysate
Lane 4: RAW264.7 cell lysate
Lane 5: C6 cell lysate
Lane 6: PC-12 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 37 kDa
Observed band size: 35 kDa

Exposure time: 20 seconds;

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 (ER00702) 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.

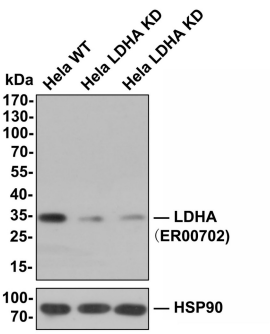


**Fig2:** All lanes: Western blot analysis of LDHA with anti-LDHA antibody (ER00702) at 1:500 dilution.
Lane 1: Wild-type Hela whole cell lysate (10 µg).
Lane 2/3: LDHA knockdown Hela whole cell lysate (10 µg).

ER00702 was shown to specifically react with LDHA in wild-type Hela cells. Weakened bands were observed when LDHA knockdown samples were tested. Wild-type and LDHA knockdown 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 (ER00702, 1:500) was used in 5% BSA 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.

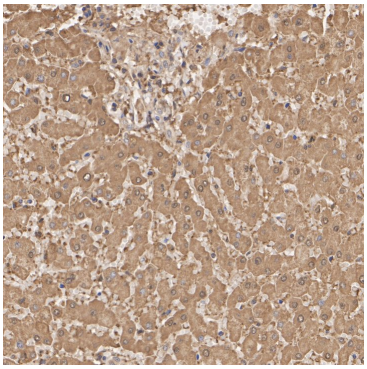

**Fig3:** Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-LDHA antibody (ER00702) at 1/1,000 dilution.

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 (ER00702) 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.

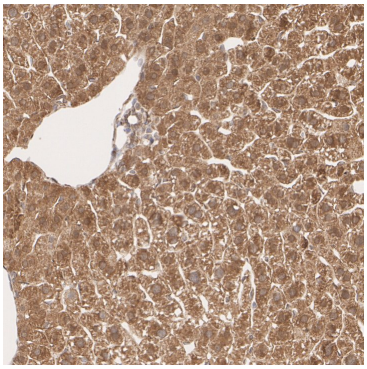

**Fig4:** Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-LDHA antibody (ER00702) at 1/1,000 dilution.

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 (ER00702) 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.

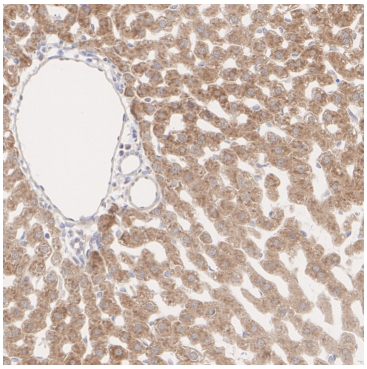

**Fig5:** Immunohistochemical analysis of paraffin-embedded rat liver tissue with Rabbit anti-LDHA antibody (ER00702) at 1/1,000 dilution.

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 (ER00702) 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.

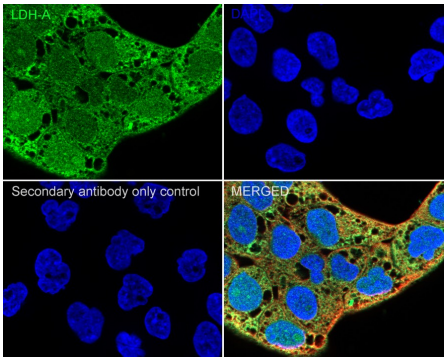


**Fig6:** Immunocytochemistry analysis of A431 cells labeling LDHA with Rabbit anti-LDHA antibody (ER00702) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-LDHA antibody (ER00702) 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 (M1305-2, 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.

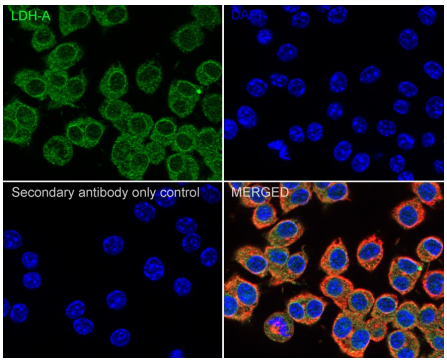


**Fig7:** Immunocytochemistry analysis of RAW264.7 cells labeling LDHA with Rabbit anti-LDHA antibody (ER00702) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-LDHA antibody (ER00702) 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 (M1305-2, 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.

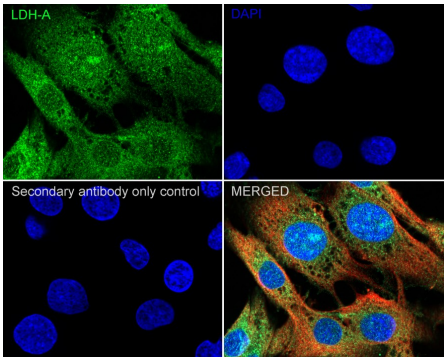


**Fig8:** Immunocytochemistry analysis of C6 cells labeling LDHA with Rabbit anti-LDHA antibody (ER00702) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-LDHA antibody (ER00702) 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 (M1305-2, 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.

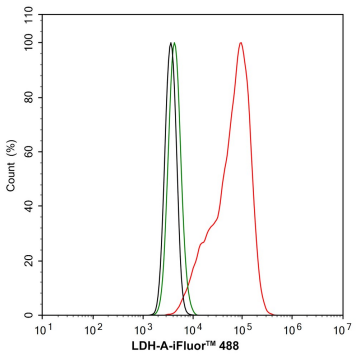


**Fig9:** Flow cytometric analysis of A431 cells labeling LDHA.

Cells were fixed and permeabilized. Then stained with the primary antibody (ER00702, 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).

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

## Background References

1. Miskimins WK et al. Synergistic anti-cancer effect of phenformin and oxamate. PLoS One 9:e85576 (2014)
2. Peng X et al. Autophagy promotes paclitaxel resistance of cervical cancer cells: involvement of Warburg effect activated hypoxia-induced factor 1-a-mediated signaling. Cell Death Dis 5:e1367 (2014)