# Anti-GAPDH Antibody [PD00-07] - Loading control HA721136

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| **Product Type:** | Recombinant Rabbit monoclonal IgG, primary antibodies |
| **Species reactivity:** | Human, Mouse, Rat, Escherichia coli, Zebrafish, Drosophila melanogaster, Rice |
| **Applications:** | WB, IHC-P, IF-Cell, FC |
| **Molecular Wt:** | Predicted band size: 36 kDa |
| **Clone number:** | PD00-07 |

**Description:** Has both glyceraldehyde-3-phosphate dehydrogenase and nitrosylase activities, thereby playing a role in glycolysis and nuclear functions, respectively. Participates in nuclear events including transcription, RNA transport, DNA replication and apoptosis. Nuclear functions are probably due to the nitrosylase activity that mediates cysteine S-nitrosylation of nuclear target proteins such as SIRT1, HDAC2 and PRKDC. Modulates the organization and assembly of the cytoskeleton. Facilitates the CHP1-dependent microtubule and membrane associations through its ability to stimulate the binding of CHP1 to microtubules. Glyceraldehyde-3-phosphate dehydrogenase is a key enzyme in glycolysis that catalyzes the first step of the pathway by converting D-glyceraldehyde 3-phosphate (G3P) into 3-phospho-D-glyceroyl phosphate. Component of the GAIT (gamma interferon-activated inhibitor of translation) complex which mediates interferon-gamma-induced transcript-selective translation inhibition in inflammation processes. Upon interferon-gamma treatment assembles into the GAIT complex which binds to stem loop-containing GAIT elements in the 3'-UTR of diverse inflammatory mRNAs (such as ceruplasmin) and suppresses their translation.

**Immunogen:** Recombinant protein within mouse GAPDH aa 94-333 / 333.

**Positive control:** HEK-293 cell lysate, HeLa cell lysate, A431 cell lysate, K-562 cell lysate, RAW264.7 cell lysate, PC-12 cell lysate, mouse heart tissue lysate, rat heart tissue lysate, human liver tissue lysate, mouse liver tissue lysate, rat liver tissue lysate, human kidney tissue lysate, mouse kidney tissue lysate, rat kidney tissue lysate, E.coli cell lysate, HepG2 cell lysates, NIH/3T3 cell lysates, PC-12 cell lysates, zebrafish tissue lysates, human pancreas tissue, human kidney tissue, mouse kidney tissue, rat kidney tissue, HeLa, NIH/3T3.

**Subcellular location:** Cytoskeleton, nucleus, cytosol, perinuclear region.

**Database links:** SwissProt: P04406 Human | P16858 Mouse | P04797 Rat

**Recommended Dilutions:**

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| **WB** | 1:2,500-1:20,000 |
| **IHC-P** | 1:1,000-1:5,000 |
| **IF-Cell** | 1:1,000 |
| **FC** | 1:1,000 |

**Storage Buffer:** PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

**Storage Instruction:** Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.

**Purity:** Protein A affinity purified.

## Images

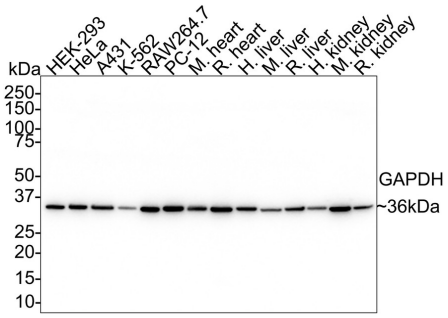


**Fig1:** Western blot analysis of GAPDH on different lysates with Rabbit anti-GAPDH antibody (HA721136) at 1/20,000 dilution.

Lane 1: HEK-293 cell lysate (20 µg/Lane)
Lane 2: HeLa cell lysate (20 µg/Lane)
Lane 3: A431 cell lysate (20 µg/Lane)
Lane 4: K-562 cell lysate (20 µg/Lane)
Lane 5: RAW264.7 cell lysate (20 µg/Lane)
Lane 6: PC-12 cell lysate (20 µg/Lane)
Lane 7: Mouse heart tissue lysate (40 µg/Lane)
Lane 8: Rat heart tissue lysate (40 µg/Lane)
Lane 9: Human liver tissue lysate (40 µg/Lane)
Lane 10: Mouse liver tissue lysate (40 µg/Lane)
Lane 11: Rat liver tissue lysate (40 µg/Lane)
Lane 12: Human kidney tissue lysate (40 µg/Lane)
Lane 13: Mouse kidney tissue lysate (40 µg/Lane)
Lane 14: Rat kidney tissue lysate (40 µg/Lane)

Predicted band size: 36 kDa
Observed band size: 36 kDa

Exposure time: 1 minute 2 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 (HA721136) at 1/20,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

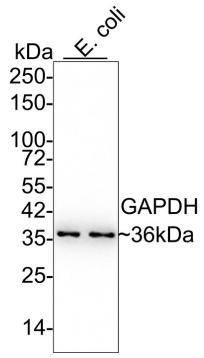


**Fig2:** Western blot analysis of GAPDH on different lysates with Rabbit anti-GAPDH antibody (HA721136) at 1/20,000 dilution.

Lane 1: E.coli cell lysate (10 µg/Lane)
Lane 2: E.coli cell lysate (no heat) (10 µg/Lane)

Notice: no heat means the lysate is not boiled.

Predicted band size: 36 kDa
Observed band size: 36 kDa

Exposure time: 30 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 (HA721136) at 1/20,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP

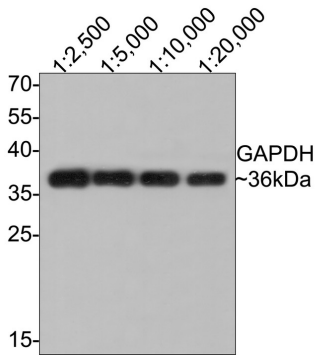


**Fig3:** Western blot analysis of GAPDH on HepG2 cell lysates with Rabbit anti-GAPDH antibody (HA721136) at different dilutions.

Lysates/proteins at 10 µg/Lane.

Predicted band size: 36 kDa
Observed band size: 36 kDa

Exposure time: 30 seconds;

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 (HA721136) at different dilutions were 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.

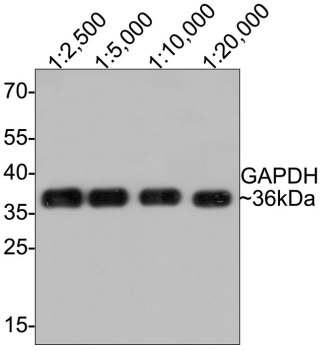


**Fig4:** Western blot analysis of GAPDH on NIH/3T3 cell lysates with Rabbit anti-GAPDH antibody (HA721136) at different dilutions.

Lysates/proteins at 10 µg/Lane.

Predicted band size: 36 kDa
Observed band size: 36 kDa

Exposure time: 30 seconds;

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 (HA721136) at different dilutions were 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.

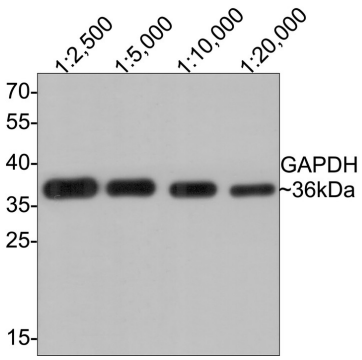


**Fig5:** Western blot analysis of GAPDH on PC-12 cell lysates with Rabbit anti-GAPDH antibody (HA721136) at different dilutions.

Lysates/proteins at 10 µg/Lane.

Predicted band size: 36 kDa
Observed band size: 36 kDa

Exposure time: 30 seconds;

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 (HA721136) at different dilutions were 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.

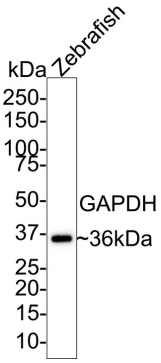


**Fig6:** Western blot analysis of GAPDH on zebrafish tissue lysates with Rabbit anti-GAPDH antibody (HA721136) at 1/20,000 dilution.

Lysates/proteins at 20 µg/Lane.

Predicted band size: 36 kDa
Observed band size: 36 kDa

Exposure time: 1 minute;

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 (HA721136) at 1/20,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

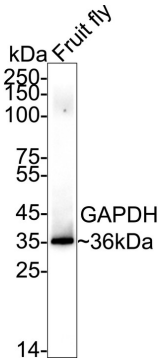


**Fig7:** Western blot analysis of GAPDH on fruit flies tissue lysates with Rabbit anti-GAPDH antibody (HA721136) at 1/10,000 dilution.

Lysates/proteins at 8 µg/Lane.

Predicted band size: 36 kDa
Observed band size: 36 kDa

Exposure time: 20 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 (HA721136) at 1/10,000 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.

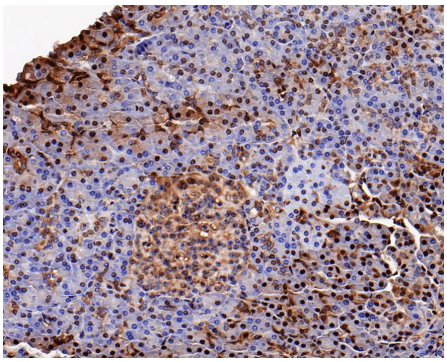

**Fig8:** Immunohistochemical analysis of paraffin-embedded human pancreas tissue with Rabbit anti-GAPDH antibody (HA721136) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with dd$H_2O$ and PBS, and then probed with the primary antibody (HA721136) 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.

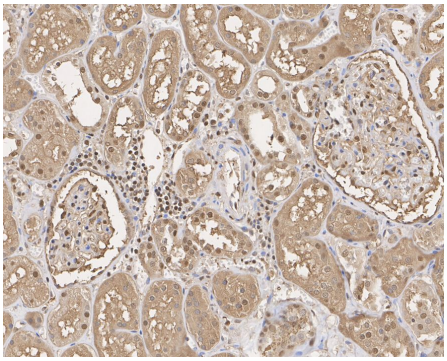

**Fig9:** Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-GAPDH antibody (HA721136) at 1/5,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with dd$H_2O$ and PBS, and then probed with the primary antibody (HA721136) at 1/5,000 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.

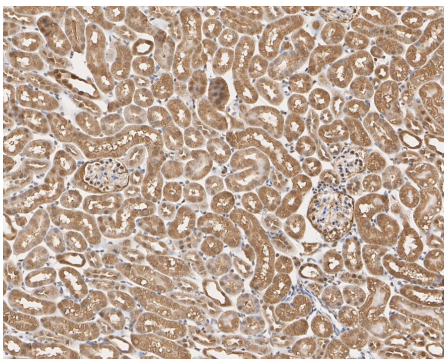

**Fig10:** Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-GAPDH antibody (HA721136) at 1/5,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with dd$H_2O$ and PBS, and then probed with the primary antibody (HA721136) at 1/5,000 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.

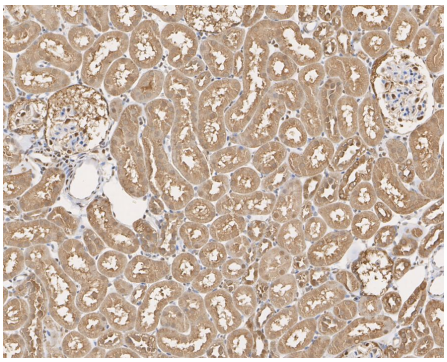

**Fig11:** Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-GAPDH antibody (HA721136) at 1/5,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with dd$H_2O$ and PBS, and then probed with the primary antibody (HA721136) at 1/5,000 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.

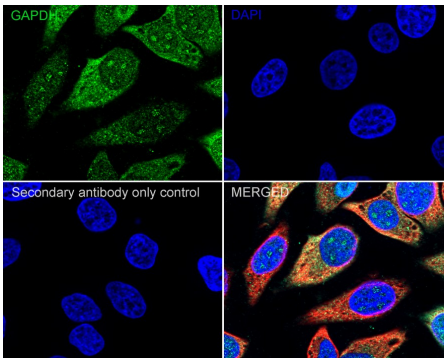


**Fig12:** Immunocytochemistry analysis of HeLa cells labeling GAPDH with Rabbit anti-GAPDH antibody (HA721136) at 1/1,000 dilution.

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-GAPDH antibody (HA721136) at 1/1,000 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.

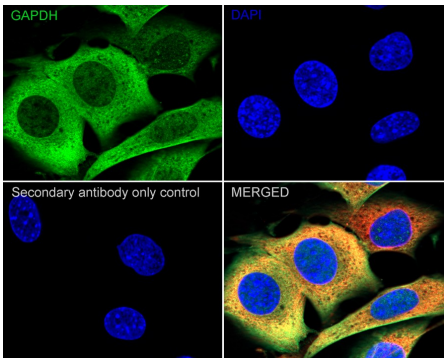


**Fig13:** Immunocytochemistry analysis of NIH/3T3 cells labeling GAPDH with Rabbit anti-GAPDH antibody (HA721136) at 1/1,000 dilution.

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-GAPDH antibody (HA721136) at 1/1,000 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.

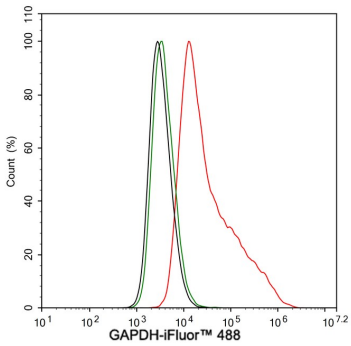


**Fig14:** Flow cytometric analysis of HeLa cells labeling GAPDH.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721136, 1μg/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. "High-resolution structure of human D-glyceraldehyde-3-phosphate dehydrogenase."Jenkins J.L., Tanner J.J.Acta Crystallogr. D 62:290-301(2006)
2. "Structural analysis of human liver glyceraldehyde-3-phosphate dehydrogenase."Ismail S.A., Park H.W.Acta Crystallogr. D 61:1508-1513(2005)