Anti-GAPDH Antibody [SA30-01] - BSA and Azide free

HA750022

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Chicken, Drosophila melanogaster, Zebrafish
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 36 kDa
Clone number:	SA30-01

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:	Rat liver tissue lysate, rat lung tissue lysate, rat heart tissue lysate, rat cerebellum tissue lysate, rat skeletal muscle tissue lysate, rat spleen tissue lysate, rat small intestine tissue lysate, hybrid fish (crucian-carp) brain tissue lysates, PC-3 cell lysate, mouse colon tissue lysate, SH-SY5Y cell lysate, NIH/3T3 cell lysate, SK-Br-3 cell lysate, rat brain tissue lysate, A549, HepG2, human liver tissue, mouse liver tissue, mouse spleen tissue, DF-1 cell lysate.
Subcellular location:	Cytoplasm, cytosol, Nucleus perinuclear region, Membrane, cytoskeleton.
Database links:	SwissProt: P04406 Human | P16858 Mouse | P04797 Rat
Recommended Dilutions:	
WB	1:50,000-1:200,000
IF-Cell	1:1,000
IF-Tissue	1:200-1:500
IHC-P	1:1,000
FC	1:1,000
IP	1-2μg/sample
Storage Buffer:	PBS (pH7.4).
Storage Instruction:	Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

Images

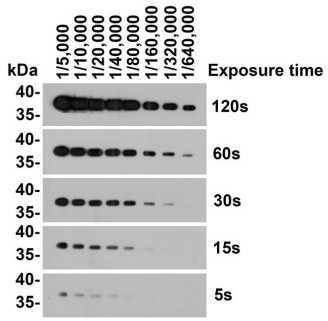

Fig1: Western blot analysis of GAPDH on Hela cell lysates with Rabbit anti-GAPDH antibody (HA750022).

Hela cell lysates at 10 µg/Lane.

Predicted band size: 36 kDa
Observed band size: 36 kDa
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 (HA750022) at serial 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.

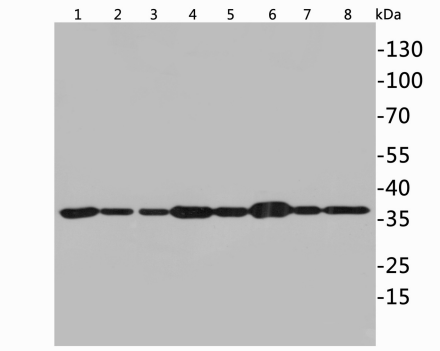

Fig2: Western blot analysis of GAPDH on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750022, 1/100,000) was used in 5% NFDM/TBST at room temperature for 1 hour. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/200,000 dilution was used for 45 mins at room temperature.

Positive control:
Lane 1: Rat liver tissue lysate, 20 µg/Lane
Lane 2: Rat lung tissue lysate, 20 µg/Lane
Lane 3: Rat lung tissue lysate, 20 µg/Lane
Lane 4: Rat heart tissue lysate, 20 µg/Lane
Lane 5: Rat cerebellum tissue lysate, 20 µg/Lane
Lane 6: Rat skeletal muscle tissue lysate, 20 µg/Lane
Lane 7: Rat spleen tissue lysate, 20 µg/Lane
Lane 8: Rat small intestine tissue lysate, 20 µg/Lane

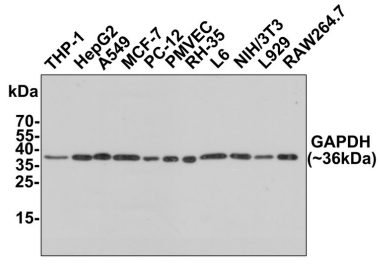

Fig3: Western blot analysis of GAPDH on different lysates with Rabbit anti-GAPDH antibody (HA750022) at 1/80,000 dilution.

Cell lysates at 10 µg/Lane.

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

Exposure time: 1 minute;
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 (HA750022) at 1/80,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit

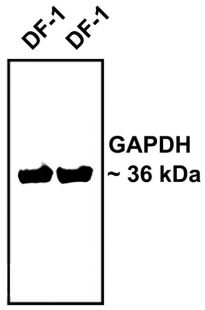

Fig4: Western blot analysis of GAPDH on DF-1 cell lysates with Rabbit anti-GAPDH antibody (HA750022) at 1/100,000 dilution.

Cell lysates at 15 µg/Lane.

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

Exposure time: 1 second;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 2 hour at room temperature. The primary antibody (HA750022) at 1/100,000 dilution was used in 5% NFDM/TBST at 4 ℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody at 1/50,000 dilution was used for 2 hour at room temperature.

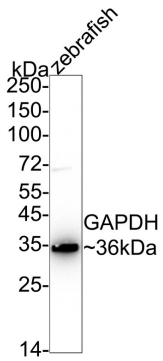

Fig5: Western blot analysis of GAPDH on zebrafish tissue lysates with Rabbit anti-GAPDH antibody (HA750022) at 1/50,000 dilution.

Lysates/proteins at 40 µg/Lane.

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

Exposure time: 14 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 (HA750022) at 1/50,000 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.

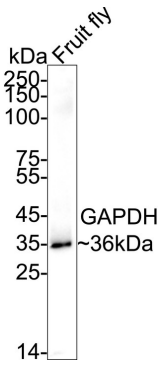

Fig6: Western blot analysis of GAPDH on fruit flies tissue lysates with Rabbit anti-GAPDH antibody (HA750022) at 1/100,000 dilution.

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 (HA750022) at 1/100,000 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.

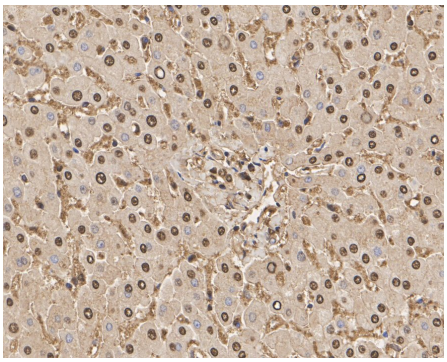

Fig7: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-GAPDH antibody (HA750022) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (HA750022) 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.

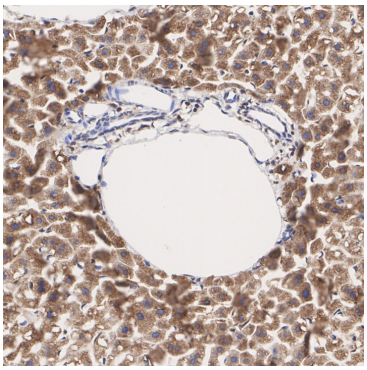

Fig8: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-GAPDH antibody (HA750022) at 1/5,000 dilution.

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 (HA750022) 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.

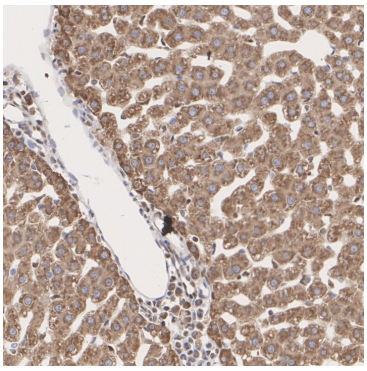

Fig9: Immunohistochemical analysis of paraffin-embedded rat liver tissue with Rabbit anti-GAPDH antibody (HA750022) at 1/5,000 dilution.

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 (HA750022) 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.

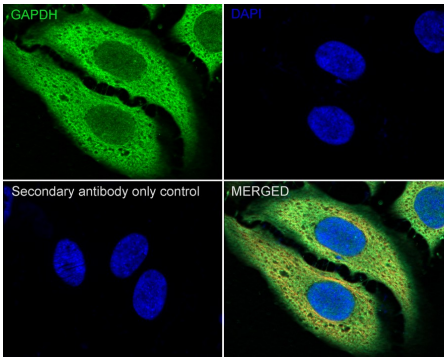

Fig10: Immunocytochemistry analysis of HeLa cells labeling GAPDH with Rabbit anti-GAPDH antibody (HA750022) at 1/1,000 dilution.

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 (HA750022) 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.

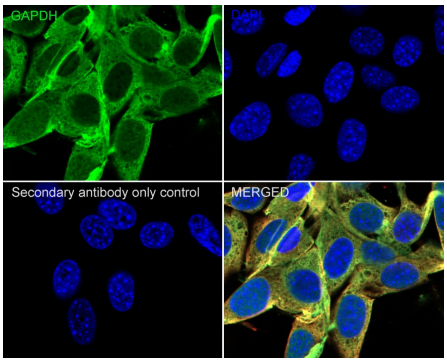

Fig11: Immunocytochemistry analysis of NIH/3T3 cells labeling GAPDH with Rabbit anti-GAPDH antibody (HA750022) at 1/2,500 dilution.

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 (HA750022) at 1/2,500 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.

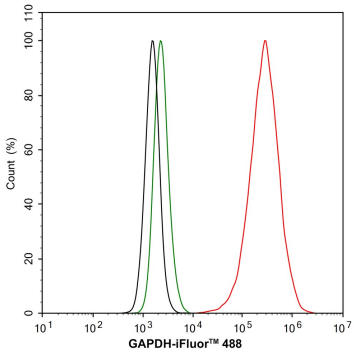

Fig12: Flow cytometric analysis of HeLa cells labeling GAPDH.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750022, 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).

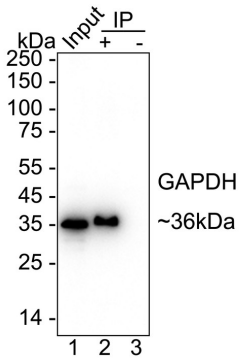

Fig13: GAPDH was immunoprecipitated from 0.2 mg A549 cell lysate with HA750022 at 2 μg/10 μl beads. Western blot was performed from the immunoprecipitate using HA750022 at 1/20,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: A549 cell lysate (input)
Lane 2: HA750022 IP in A549 cell lysate
Lane 3: Rabbit IgG instead of HA750022 in A549 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 7 seconds; ECL: K1801

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)