

Anti-GAPDH Antibody [SA30-01] - Loading control

ET1601-4



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Chicken, 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, Nucleus, Membrane

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

Recommended Dilutions:

WB	1:5,000-1:640,000
IF-Cell	1:50
IF-Tissue	1:50
IHC-P	1:50
FC	1:50
IP	Use at an assay dependent concentration.

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4°C after thawing. Aliquot store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

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Images

Fig1: Western blot analysis of GAPDH on HeLa cell lysates with Rabbit anti-GAPDH antibody (ET1601-4).

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 (ET1601-4) 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.

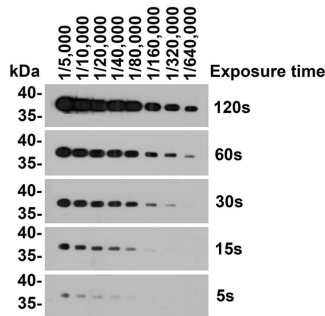
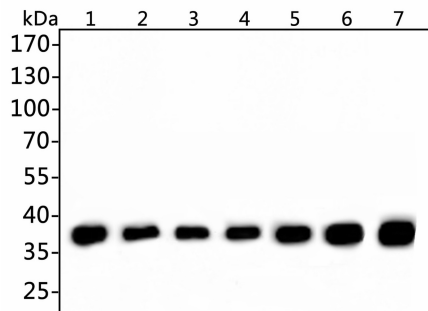


Fig2: Western blot analysis of GAPDH on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% NFDm/TBST for 1 hour at room temperature. The primary antibody (ET1601-4, 1/5,000) was used in 5% NFDm/TBST at room temperature for 1 hour. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 45 mins at room temperature.



Positive control:

Lane 1: PC-3 cell lysate, 10 µg/Lane

Lane 2: Mouse colon tissue lysate, 20 µg/Lane

Lane 3: SH-SY5Y cell lysate, 10 µg/Lane

Lane 4: PC-3 cell lysate, 10 µg/Lane

Lane 5: NIH/3T3 cell lysate, 10 µg/Lane

Lane 6: SK-Br-3 cell lysate, 10 µg/Lane

Lane 7: Rat brain tissue lysate, 20 µg/Lane

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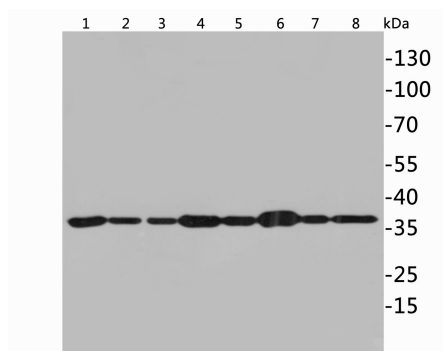


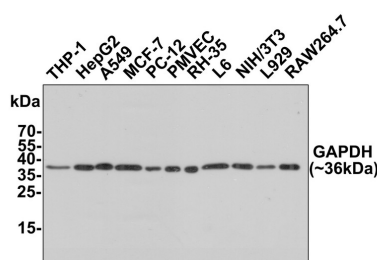
Fig3: Western blot analysis of GAPDH on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1601-4, 1/10,000) was used in 5% NFDM/TBST at room temperature for 1 hour. Goat Anti-Rabbit IgG - HRP Secondary 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

Fig4: Western blot analysis of GAPDH on different lysates with Rabbit anti-GAPDH antibody (ET1601-4) at 1/80,000 dilution.

Cell lysates at 10 µg/Lane, tissue lysates at 20 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 (ET1601-4) at 1/80,000 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.

Fig5: Western blot analysis of GAPDH on DF-1 cell lysates with Rabbit anti-GAPDH antibody (ET1601-4) at 1/10,000 dilution.

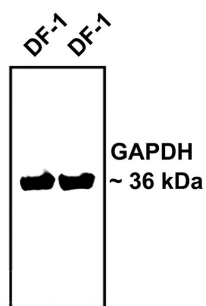
Cell lysates at 15 µg/Lane.

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 (ET1601-4) at 1/10,000 dilution was used in 5% NFDM/TBST at 4 °C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody at 1:5,000 dilution was used for 2 hour at room temperature.

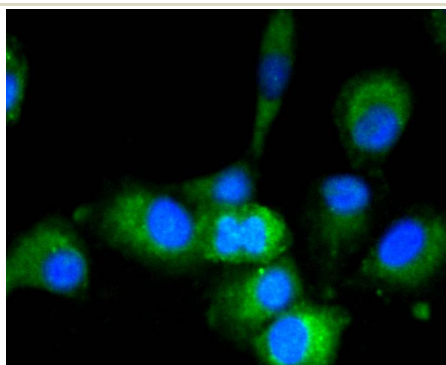


Fig6: ICC staining of GAPDH in A549 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1601-4, 1/50) for 1 hour at room 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).

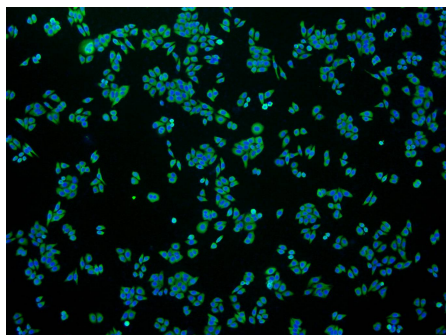


Fig7: ICC staining of GAPDH in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1601-4, 1/50) for 1 hour at room 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).

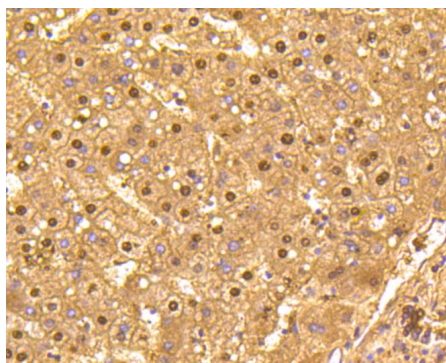


Fig8: Immunohistochemical analysis of paraffin-embedded human liver tissue using anti-GAPDH antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1601-4, 1/50) for 30 minutes 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.

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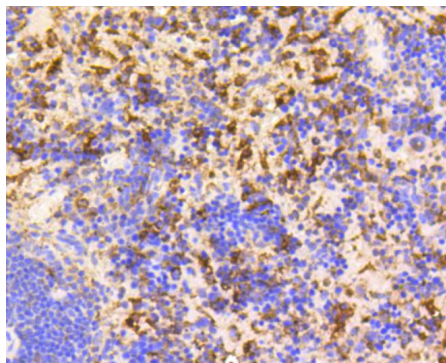


Fig9: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue using anti-GAPDH antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1601-4, 1/50) for 30 minutes 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.

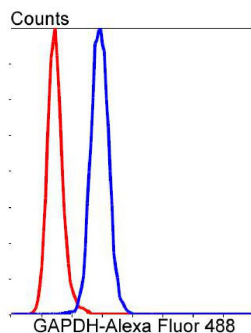


Fig10: Flow cytometric analysis of GAPDH was done on HepG2 cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1601-4, 1/50) (blue). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat 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; red).

Fig11: Western blot analysis of GAPDH on zebrafish tissue lysates with Rabbit anti-GAPDH antibody (ET1601-4) at 1/5,000 dilution.

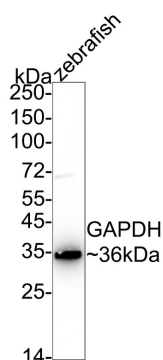
Lysates/proteins at 40 µg/Lane.

Predicted band size: 36 kDa

Observed band size: 36 kDa

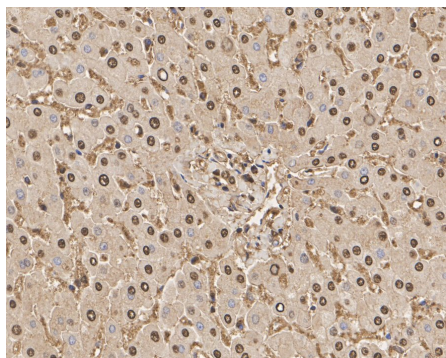
Exposure time: 14 seconds;

4-20% SDS-PAGE gel.



Proteins were transferred to a PVDF membrane and blocked with 5% NFDN/TBST for 1 hour at room temperature. The primary antibody (ET1601-4) at 1/5,000 dilution was used in 5% NFDN/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig12: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-GAPDH antibody (ET1601-4) 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 ddH₂O and PBS, and then probed with the primary antibody (ET1601-4) 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

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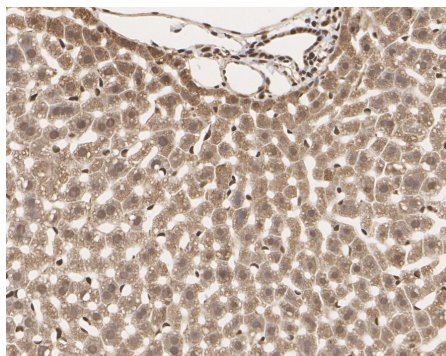


Fig13: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-GAPDH antibody (ET1601-4) at 1/1,000 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (ET1601-4) 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.

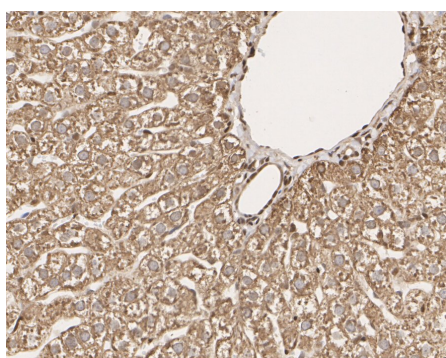
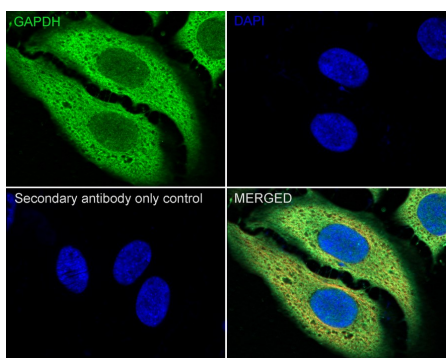


Fig14: Immunohistochemical analysis of paraffin-embedded rat liver tissue with Rabbit anti-GAPDH antibody (ET1601-4) at 1/1,000 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (ET1601-4) 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.

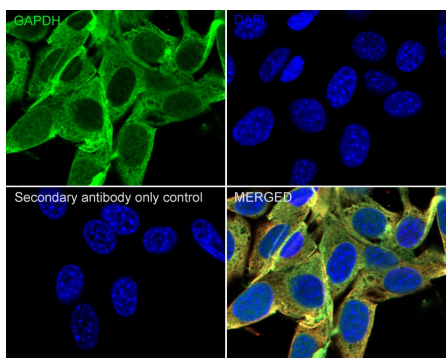
Fig15: Immunocytochemistry analysis of HeLa cells labeling GAPDH with Rabbit anti-GAPDH antibody (ET1601-4) at 1/100 dilution.



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-GAPDH antibody (ET1601-4) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. 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 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Fig16: Immunocytochemistry analysis of NIH/3T3 cells labeling GAPDH with Rabbit anti-GAPDH antibody (ET1601-4) 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 (ET1601-4) at 1/2,500 dilution in 1% BSA in PBST overnight at 4 °C. 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

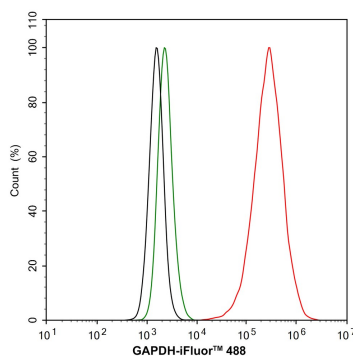


Fig17: Flow cytometric analysis of HeLa cells labeling GAPDH.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1601-4, 1µg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C 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°C. 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)

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