Anti-GAPDH Antibody [12D6]

M1310-2

Product Type:	Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat, Zebrafish, Escherichia coli
Applications:	WB, IF-Cell, IHC-P
Molecular Wt:	Predicted band size: 36 kDa
Clone number:	12D6

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 and suppresses their translation.

Immunogen: Synthetic peptide within human GAPDH aa 180-220.

Positive control: Hela cell lysate, A549 cell lysate, HepG2 cell lysate, PC-12 cell lysate, F9 cell lysate, Escherichia coli lysate, D3, A431, zebrafish tissue lysates, human thyroid carcinoma tissue, human colon carcinoma tissue.

Subcellular location: Cytoplasm, Nucleus, Membrane.

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

Recommended Dilutions:

WB	1:2000-1:5,000
IF-Cell	1:200
IHC-P	1:600

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 40% 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: Protein G affinity purified.

Images

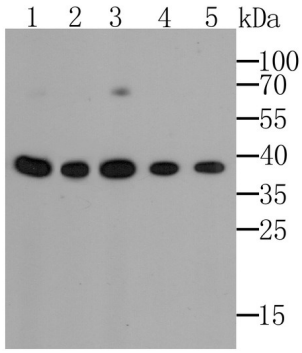

Fig1: Western blot analysis of GAPDH on different cells lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (M1310-2, 1/1000) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:5,000 dilution was used for 1 hour at room temperature.
Positive control:
Lane 1: Hela cell lysate
Lane 2: A549 cell lysate
Lane 3: HepG2 cell lysate
Lane 4: PC-12 cell lysate
Lane 5: F9 cell lysate

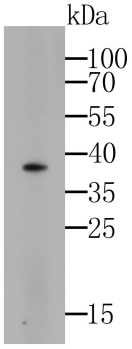

Fig2: Western blot analysis of GAPDH on zebrafish tissue lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (M1310-2, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:5,000 dilution was used for 1 hour at room temperature.

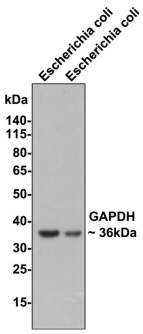

Fig3: Western blot analysis of GAPDH on different lysates with Mouse anti-GAPDH antibody (M1310-2) at 1/500 dilution.

Lane 1: Escherichia coli lysate
Lane 2: Escherichia coli lysate

Lysates/proteins at 10 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 (M1310-2) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:150,000 dilution was used for 1 hour at room temperature.

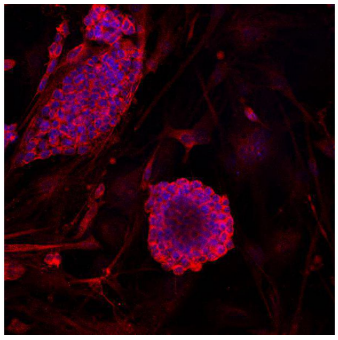

Fig4: ICC staining of GAPDH in D3 cells (red). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (M1310-2, 1/100) for 1 hour at room temperature, washed with PBS. Alexa Fluor®555 Goat anti-Mouse IgG was used as the secondary antibody at 1/100 dilution. The nuclear counter stain is DAPI (blue).

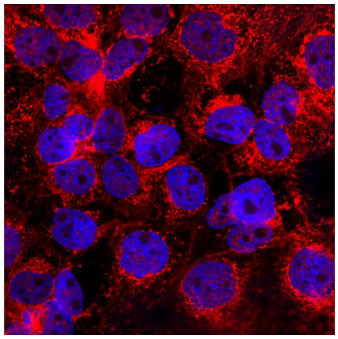

Fig5: ICC staining of GAPDH in A431 cells (red). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (M1310-2, 1/100) for 1 hour at room temperature, washed with PBS. Alexa Fluor®555 Goat anti-Mouse IgG was used as the secondary antibody at 1/100 dilution. The nuclear counter stain is DAPI (blue).

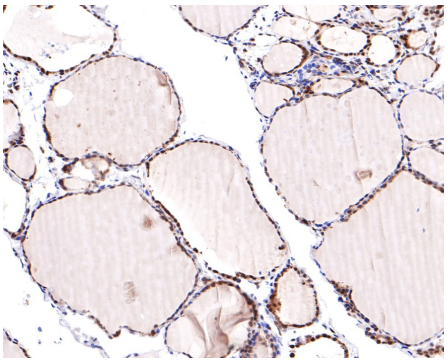

Fig6: Immunohistochemical analysis of paraffin-embedded human thyroid carcinoma tissue with Mouse anti-GAPDH antibody (M1310-2) at 1/600 dilution.

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 (M1310-2) at 1/600 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.

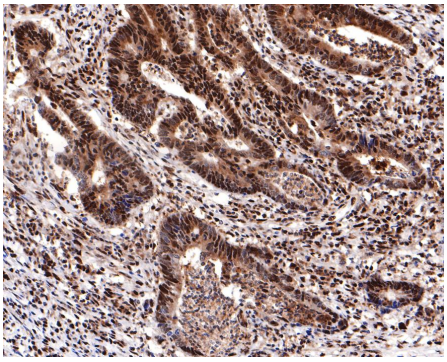

Fig7: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Mouse anti-GAPDH antibody (M1310-2) at 1/600 dilution.

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 (M1310-2) at 1/600 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.

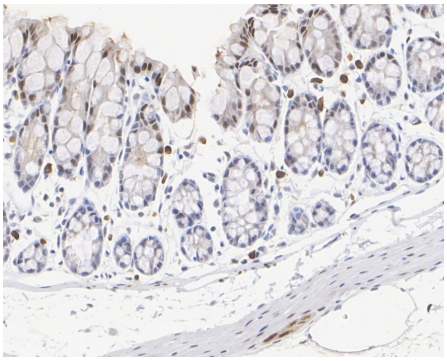

Fig8: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Mouse anti-GAPDH antibody (M1310-2) at 1/1,000 dilution.

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 (M1310-2) 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.

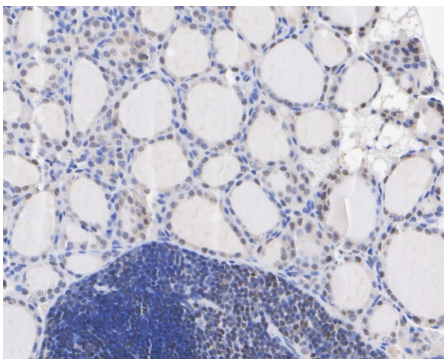

Fig9: Immunohistochemical analysis of paraffin-embedded mouse thyroid tissue with Mouse anti-GAPDH antibody (M1310-2) at 1/1,000 dilution.

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 (M1310-2) 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.

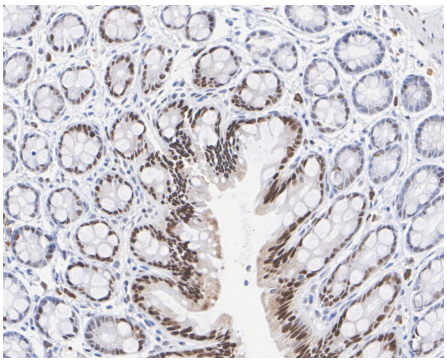

Fig10: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Mouse anti-GAPDH antibody (M1310-2) at 1/1,000 dilution.

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 (M1310-2) 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.

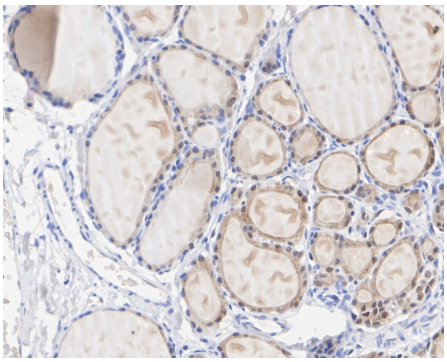

Fig11: Immunohistochemical analysis of paraffin-embedded rat thyroid tissue with Mouse anti-GAPDH antibody (M1310-2) at 1/1,000 dilution.

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 (M1310-2) 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.

Background References

1. Cheng, Y., Hou, T., Ping, J., Chen, T., & Yin, B. (2018). LMO3 promotes hepatocellular carcinoma invasion, metastasis and anoikis inhibition by directly interacting with LATS1 and suppressing Hippo signaling. J Exp Clin Cancer Res, 37(1), 228.
2. Liu, J., Wang, Y., Song, L., Zeng, L., Yi, W., Liu, T., . . . Cong, Y. S. (2017). A critical role of DDRGK1 in endoplasmic reticulum homoeostasis via regulation of IRE1alpha stability. Nat Commun, 8, 14186.
3. Jiang, Y., Tian, M., Lin, W., Wang, X., & Wang, X. (2018). Protein Kinase Serine/Threonine Kinase 24 Positively Regulates Interleukin 17-Induced Inflammation by Promoting IKK Complex Activation. Front Immunol, 9, 921.
4. Bin, G., Jiarong, Z., Shihao, W., Xiuli, S., Cheng, X., Liangbiao, C., & Ming, Z. (2012). Aire promotes the self-renewal of embryonic stem cells through Lin28. Stem Cells Dev, 21(15), 2878-2890.
5. Liu, J., Wang, Y., Song, L., et al. Cong, Y. S. (2017). A critical role of DDRGK1 in endoplasmic reticulum homoeostasis via regulation of IRE1alpha stability. Nat Commun, 8, 14186. doi: 10.1038/ncomms14186
6. Zhang, D., Zhao, Q., Sun, H., Yin, L., Wu, J., Xu, J., . . . Liang, C. (2016). Defective autophagy leads to the suppression of stem-like features of CD271(+) osteosarcoma cells. J Biomed Sci, 23(1), 82. doi: 10.1186/s12929-016-0297-5