Anti-GAPDH Antibody [12D7]

EM1901-57

Product Type:	Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat, Zebrafish
Applications:	WB, IHC-P, FC
Molecular Wt:	Predicted band size: 36 kDa
Clone number:	12D7

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 (By similarity). 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: Synthetic peptide within Human GAPDH aa 174-223 / 335.

Positive control: Hep G2 (Human hepatocellular carcinoma cell), HEK-293 (Human embryonic kidney cell), F9 (Mouse teratocarcinoma cell), PC-12 (Rat pheochromocytoma cell (undifferentiated)), Mouse heart tissue lysate,Rat heart tissue lysate,human tonsil tissue, human colon carcinoma tissue, human esophagus tissue, human small intestine tissue, human pancreas tissue, A549.

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

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

Recommended Dilutions:

WB	1:1,000-1:5,000
IHC-P	1:50-1:100
FC	1:50-1:100

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 50% 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: Protein G affinity purified.

Images

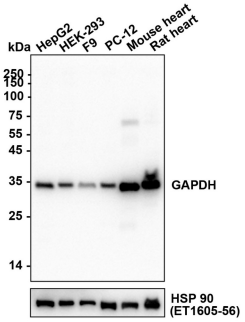

Fig1: Western blot analysis of GAPDH on different lysates with Rabbit anti-GAPDH antibody (EM1901-57) at 1/5,000 dilution.

Lane 1: Hep G2 (Human hepatocellular carcinoma cell)
Lane 2: HEK-293 (Human embryonic kidney cell)
Lane 3: F9 (Mouse teratocarcinoma cell)
Lane 4: PC-12 (Rat pheochromocytoma cell (undifferentiated))
Lane 5: Mouse heart tissue lysate
Lane 6: Rat heart tissue lysate

Lysates/proteins at 15 µg/Lane.
Exposure time: 12 seconds; ECL: K1801

Blocking: 5% NFDM/TBST, 1 hour at room temperature
Primary antibody: EM1901-57, 1/5,000 in primary antibody dilution buffer (K1803), overnight at 4 ℃
Secondary antibody: Goat anti-Mouse IgG-HRP (HA1006), 1/50,000 in 5% NFDM/TBST, 1 hour at room temperature

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

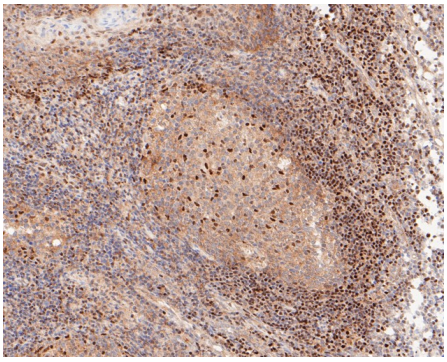

Fig2: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-GAPDH antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM1901-57, 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.

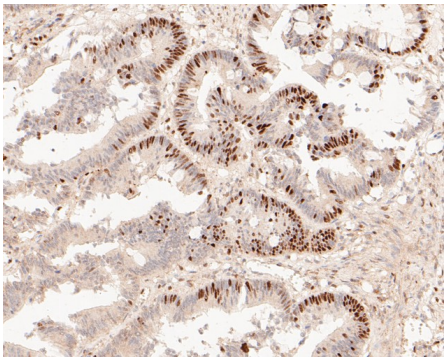

Fig3: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue using anti-GAPDH antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM1901-57, 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.

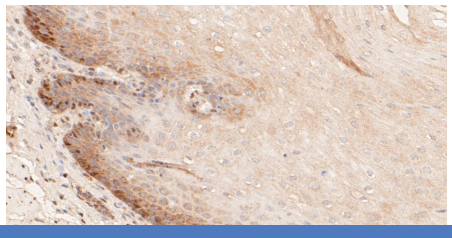

Fig4: Immunohistochemical analysis of paraffin-embedded human esophagus tissue using anti-GAPDH antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary

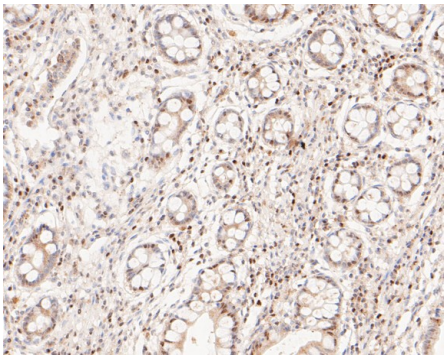

Fig5: Immunohistochemical analysis of paraffin-embedded human small intestine tissue using anti-GAPDH antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM1901-57, 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.

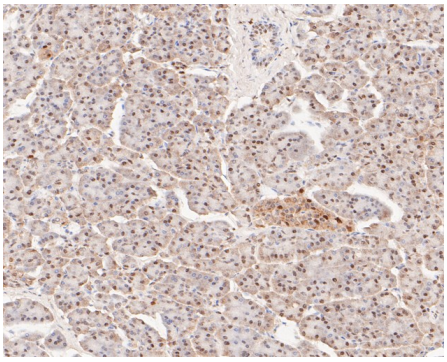

Fig6: Immunohistochemical analysis of paraffin-embedded human pancreas tissue using anti-GAPDH antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM1901-57, 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.

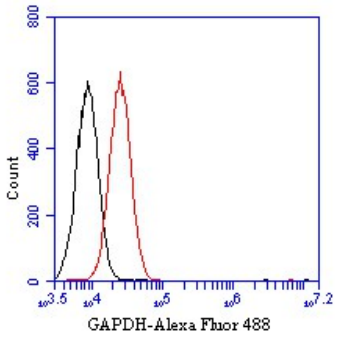

Fig7: Flow cytometric analysis of GAPDH was done on A549 cells. The cells were fixed, permeabilized and stained with the primary antibody (EM1901-57, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Mouse IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

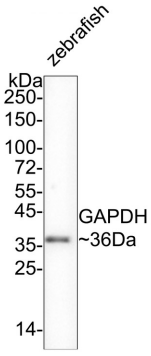

Fig8: Western blot analysis of GAPDH on zebrafish tissue lysates with Mouse anti-GAPDH antibody (EM1901-57) at 1/2,000 dilution.

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 2 minutes;

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 (EM1901-57) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

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

Background References

1. Kosova AA.et. al. Role of Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) in DNA Repair. Biochemistry (Mosc). 2017 Jun;82(6):643-654.
2. Nicholls C.et. al. GAPDH: a common enzyme with uncommon functions. Clin Exp Pharmacol Physiol. 2012 Aug;39(8):674-9.