Anti-GAPDH Antibody [5-E10]

EM1101

Product Type:	Mouse monoclonal IgM, primary antibodies
Species reactivity:	Human, Mouse, Rat, Zebrafish, Rabbit
Applications:	WB, IF-Cell, IHC-P
Molecular Wt:	Predicted band size: 36 kDa
Clone number:	5-E10

Description: GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) has both glyceraldehyde-3-phosphate dehydrogenase and nitrosylase activities, thereby playing a role in glycolysis and nuclear functions, respectively. It participates in nuclear events including transcription, RNA transport, DNA replication and apoptosis. GAPDH 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.

Immunogen: Synthetic peptide within human GAPDH aa 10-50.

Positive control: MDA-MB-231 cell lysate, HeLa cell lysate, MCF7 cell lysate, SK-Br-3 cell lysate, JAR cell lysate, NIH/3T3 cell lysate, C2C12 cell lysate, PC-12 cell lysate, mouse kidney tissue lysate, rat liver tissue lysate, F9, Hela, HepG2.

Subcellular location: Cytoplasm

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

Recommended Dilutions:

WB	1:5,000-1:10,000
IF-Cell	1:200-1:500
IHC-P	1:200-1:1,000

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 L affinity purified.

Images

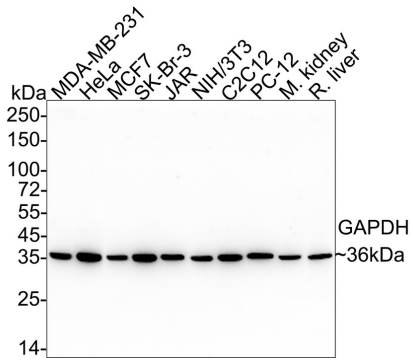


Fig1: Western blot analysis of GAPDH on different lysates with Mouse anti-GAPDH antibody (EM1101) at 1/5,000 dilution.

Lane 1: MDA-MB-231 cell lysate (20 µg/Lane)
Lane 2: HeLa cell lysate (20 µg/Lane)
Lane 3: MCF7 cell lysate (20 µg/Lane)
Lane 4: SK-Br-3 cell lysate (20 µg/Lane)
Lane 5: JAR cell lysate (20 µg/Lane)
Lane 6: NIH/3T3 cell lysate (20 µg/Lane)
Lane 7: C2C12 cell lysate (20 µg/Lane)
Lane 8: PC-12 cell lysate (20 µg/Lane)
Lane 9: Mouse kidney tissue lysate (40 µg/Lane)
Lane 10: Rat liver tissue lysate (40 µg/Lane)

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

Exposure time: 1 minute 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 (EM1101) at 1/5,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

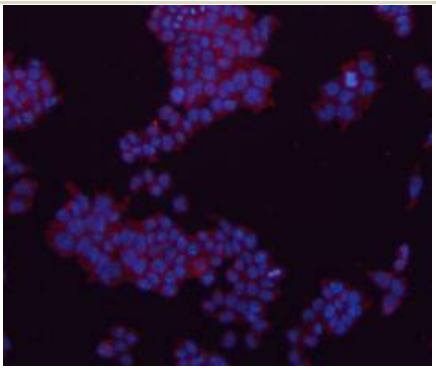


Fig2: ICC staining GAPDH in F9 cells (red). The nuclear counter stain is DAPI (blue). Cells were fixed in paraformaldehyde, permeabilised with 0.25% Triton X100/PBS.

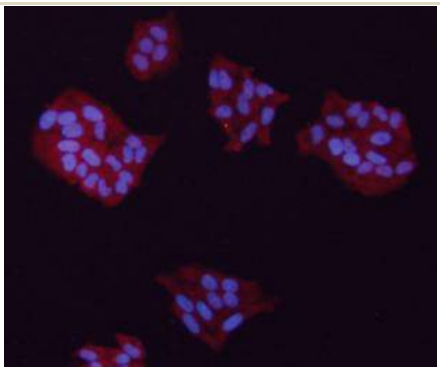


Fig3: ICC staining GAPDH in Hela cells (red). The nuclear counter stain is DAPI (blue). Cells were fixed in paraformaldehyde, permeabilised with 0.25% Triton X100/PBS.

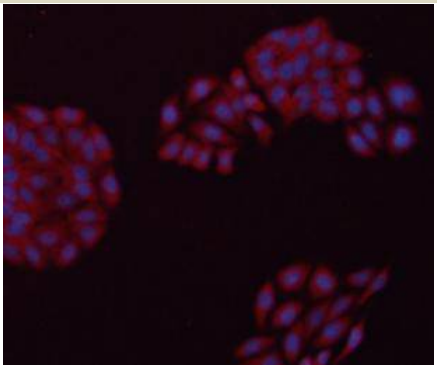
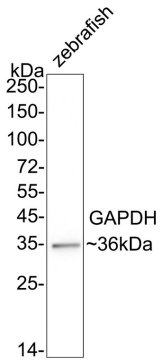


Fig4: ICC staining GAPDH in HepG2 cells (red). The nuclear counter stain is DAPI (blue). Cells were fixed in paraformaldehyde, permeabilised with 0.25% Triton X100/PBS.

Fig5: Western blot analysis of GAPDH on zebrafish cell/tissue lysates with Mouse anti-GAPDH antibody (EM1101) at 1/5,000 dilution.



Lysates/proteins at 20 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 (EM1101) at 1/5,000 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.

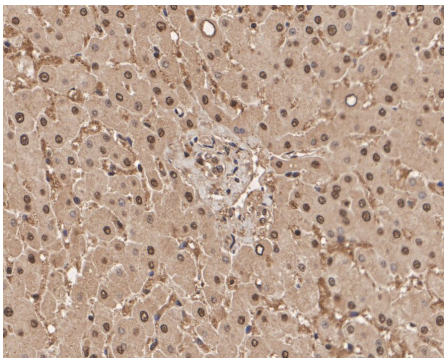


Fig6: Immunohistochemical analysis of paraffin-embedded human liver tissue with Mouse anti-GAPDH antibody (EM1101) at 1/200 dilution.

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 (EM1101) 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.

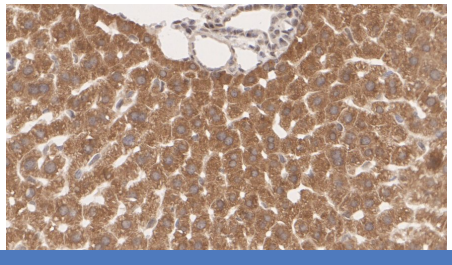


Fig7: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Mouse anti-GAPDH antibody (EM1101) at 1/200 dilution.

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

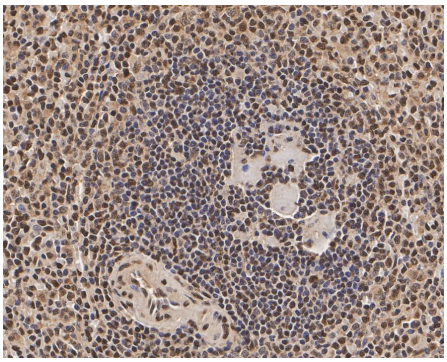


Fig8: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Mouse anti-GAPDH antibody (EM1101) at 1/200 dilution.

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 (EM1101) 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.

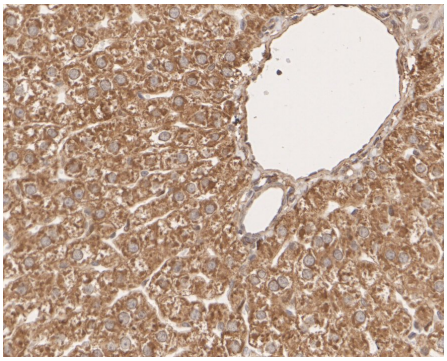


Fig9: Immunohistochemical analysis of paraffin-embedded rat liver tissue with Mouse anti-GAPDH antibody (EM1101) at 1/200 dilution.

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 (EM1101) 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.

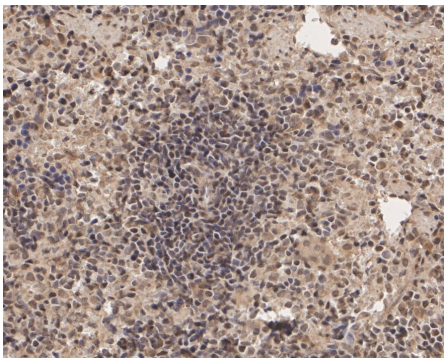


Fig10: Immunohistochemical analysis of paraffin-embedded rat spleen tissue with Mouse anti-GAPDH antibody (EM1101) at 1/200 dilution.

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 (EM1101) 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.

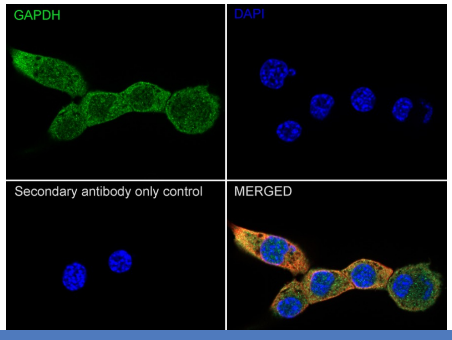


Fig11: Immunocytochemistry analysis of F9 cells labeling GAPDH with Mouse anti-GAPDH antibody (EM1101) at 1/100 dilution.

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 Mouse anti-GAPDH antibody (EM1101) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) 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.

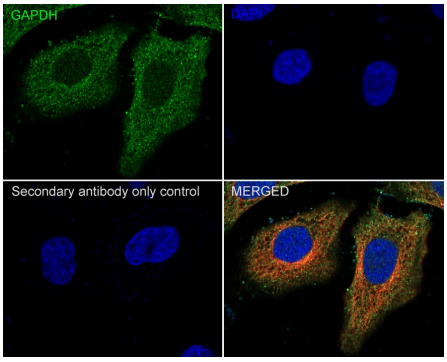


Fig12: Immunocytochemistry analysis of HeLa cells labeling GAPDH with Mouse anti-GAPDH antibody (EM1101) at 1/100 dilution.

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 Mouse anti-GAPDH antibody (EM1101) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) 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 (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

Background References

1. "The glyceraldehyde 3 phosphate dehydrogenase gene family: structure of a human cDNA and of an X chromosome linked pseudogene; amazing complexity of the gene family in mouse." Hanauer A., Mandel J.-L. EMBO J. 3:2627-2633(1983)
2. "Enhanced expression of a glyceraldehyde-3-phosphate dehydrogenase gene in human lung cancers." Tokunaga K., Nakamura Y., Sakata K., Fujimori K., Ohkubo M., Sawada K., Sakiyama S. Cancer Res. 47:5616-5619(1986)
3. "Glyceraldehyde-3-phosphate dehydrogenase is phosphorylated by protein kinase Ciota /lambda and plays a role in microtubule dynamics in the early secretory pathway." Tisdale E.J.J. Biol. Chem. 277:3334-3341(2001)