Anti-GAPDH Antibody

ER1901-65

Product Type:	Rabbit polyclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, FC
Molecular Wt:	36 kDa

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 inflammatio n 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 Human GAPDH aa 15-171.

Positive control: PC-12 cell lysate, Hela cell lysate, mouse ovary tissue lysate, human placenta tissue lysate, rat brain tissue lysate, NCCIT cell lysate, A549, LoVo, MCF-7, rat kidney tissue, human colon cancer tissue, human spleen tissue, mouse testis tissue.

Subcellular location: Cytoplasm, Nucleus, Membrane.

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

Recommended Dilutions:

WB	1:500-1:2,000
IF-Cell	1:50-1:200
IHC-P	1:50-1:200
FC	1:50-1:100

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.

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

Purity: Protein A affinity purified.

Images

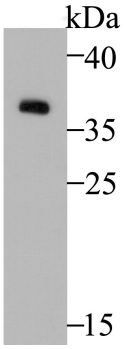

Fig1: Western blot analysis of GAPDH on PC-12 cell lysate. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody was used at a 1:2,000 dilution in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.

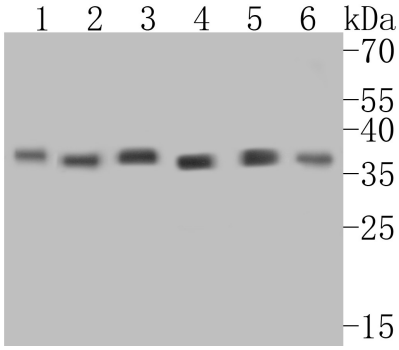

Fig2: Western blot analysis of GAPDH on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ER1901-65, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.
Positive control:
Lane 1: PC-12 cell lysate
Lane 2: Hela cell lysate
Lane 3: Mouse ovary tissue lysate
Lane 4: Human placenta tissue lysate
Lane 5: Rat brain tissue lysate
Lane 6: NCCIT cell lysate

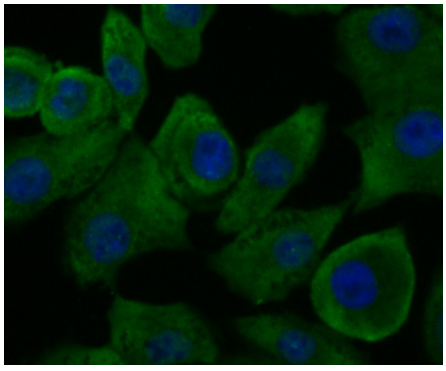

Fig3: ICC staining of GAPDH in A549 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the antibody (ER1901-65) at a dilution of 1:50 for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/100 dilution. The nuclear counter stain is DAPI (blue).

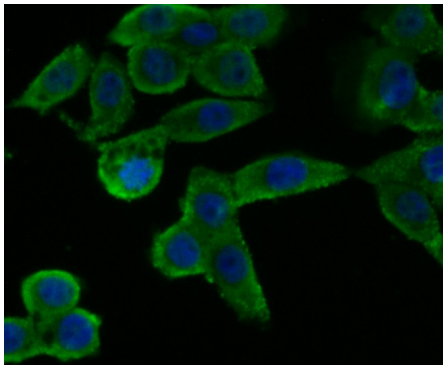

Fig4: ICC staining of GAPDH in LoVo cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the antibody (ER1901-65) at a dilution of 1:50 for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/100 dilution. The nuclear counter stain is DAPI (blue).

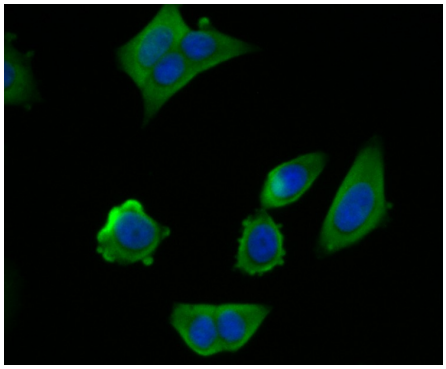

Fig5: ICC staining of GAPDH in MCF-7 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the antibody (ER1901-65) at a dilution of 1:100 for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/100 dilution. The nuclear counter stain is DAPI (blue).

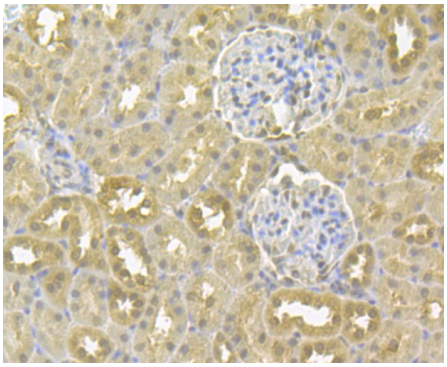

Fig6: Immunohistochemical analysis of paraffin-embedded rat kidney tissue using anti-GAPDH antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the antibody (ER1901-65) at 1/50 dilution, for 30 minutes at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. Counter stained with hematoxylin and mounted with DPX.

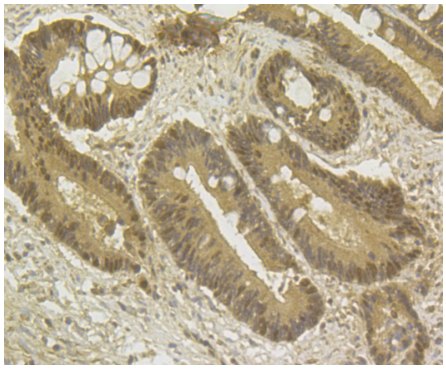

Fig7: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue using anti-GAPDH antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the antibody (ER1901-65) at 1/50 dilution, for 30 minutes at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. Counter stained with hematoxylin and mounted with DPX.

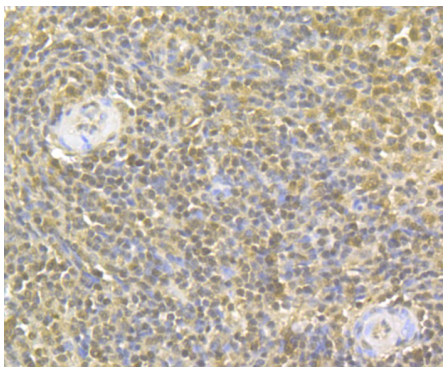

Fig8: Immunohistochemical analysis of paraffin-embedded human spleen tissue using anti-GAPDH antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the antibody (ER1901-65) at 1/50 dilution, for 30 minutes at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. Counter stained with hematoxylin and mounted with DPX.

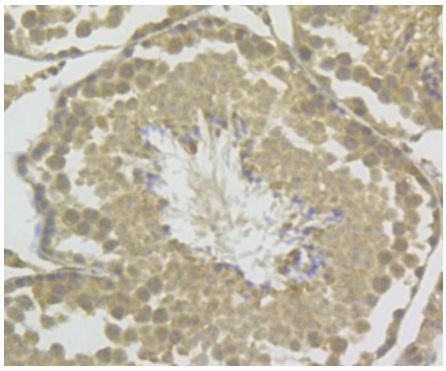

Fig9: Immunohistochemical analysis of paraffin-embedded mouse testis tissue using anti-GAPDH antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the antibody (ER1901-65) at 1/50 dilution, for 30 minutes at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. Counter stained with hematoxylin and mounted with DPX.

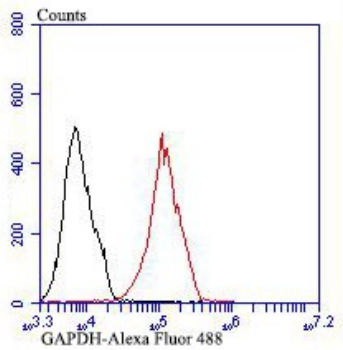

Fig10: Flow cytometric analysis of GAPDH was done on MCF-7 cells. The cells were fixed, permeabilized and stained with GAPDH antibody at 1/50 dilution (red) compared with an unlabelled control (cells without incubation with primary antibody; black). After incubation of the primary antibody on room temperature for an hour, the cells was stained with a Alexa Fluor 488-conjugated goat anti-rabbit IgG Secondary antibody at 1/500 dilution for 30 minutes.

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

Background References

1. Jenkins JL et al. High-resolution structure of human D-glyceraldehyde-3-phosphate dehydrogenase. Acta Crystallogr. D 62:290-301 (2006).
2. Ismail SA et al. Structural analysis of human liver glyceraldehyde-3-phosphate dehydrogenase. Acta Crystallogr. D 61:1508-1513 (2005).