Anti-GAPDH Antibody

R1210-1

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

Description: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) catalyzes the phosphorylation of glyceraldehyde-3-phosphate during glycolysis. It participates in nuclear events including transcription, RNA transport, DNA replication and apoptosis. GAPDH is thought to be a constitutively expressed housekeeping protein. For this reason, GAPDH mRNA and protein levels are often measured as controls in experiments quantifying specific changes in expression of other targets.

Immunogen: This antibody is produced by immunizing rabbits with full length recombinant protein of GAPDH.

Positive control: HeLa cell lysate, A431 cell lysate, HEK-293 cell lysate, Jurkat cell lysate, NIH/3T3 cell lysate, RAW264.7 cell lysate, PC-12 cell lysate, C6 cell lysate, mouse brain tissue lysate, rat brain tissue lysate, A549, LOVO, MCF-7, rat kidney tissue, human colon cancer tissue, human spleen tissue, mouse testis tissue, Hela.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: P04406 Human | P16858 Mouse | P46406 Rabbit

Recommended Dilutions:

WB	1:20,000
IF-Cell	1:100-1:200
IHC-P	1:50-1:200
FC	1:50-1:100

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℃. Avoid repeated freeze / thaw cycles.

Purity: Immunogen affinity purified.

Images

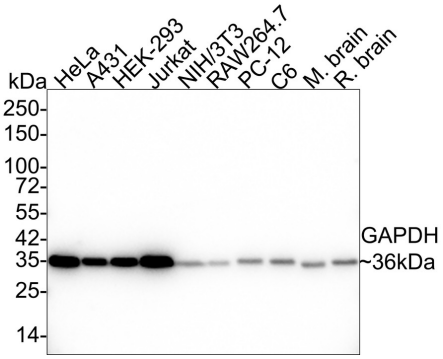

Fig1: Western blot analysis of GAPDH on different lysates with Rabbit anti-GAPDH antibody (R1210-1) at 1/20,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: A431 cell lysate
Lane 3: HEK-293 cell lysate
Lane 4: Jurkat cell lysate
Lane 5: NIH/3T3 cell lysate
Lane 6: RAW264.7 cell lysate
Lane 7: PC-12 cell lysate
Lane 8: C6 cell lysate
Lane 9: Mouse brain tissue lysate
Lane 10: Rat brain tissue lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 20 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 (R1210-1) at 1/20,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

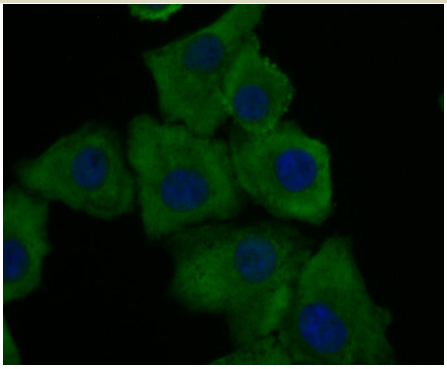

Fig2: ICC staining GAPDH in A549 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the antibody (R1210-1) at a dilution of 1:100 for 1 hour at room temperature, washed with PBS. Alexa Fluorc™ 488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/100 dilution. The nuclear counter stain is DAPI (blue).

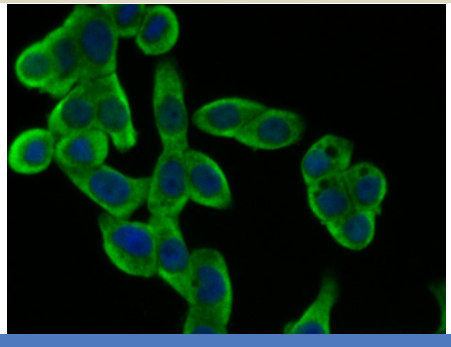

Fig3: ICC staining GAPDH in LOVO cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the antibody (R1210-1) at a dilution of 1:100 for 1 hour at room temperature, washed with PBS. Alexa Fluorc™ 488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/100 dilution. The nuclear counter stain is DAPI (blue).

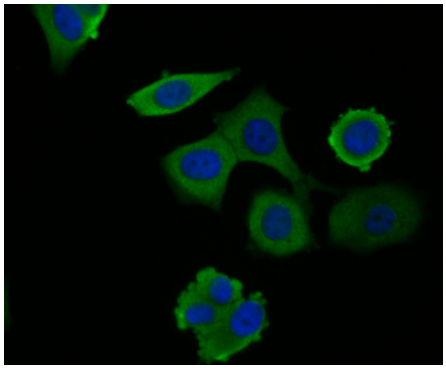

Fig4: ICC staining GAPDH in MCF-7 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the antibody (R1210-1) at a dilution of 1:100 for 1 hour at room temperature, washed with PBS. Alexa Fluorc™ 488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/100 dilution. The nuclear counter stain is DAPI (blue).

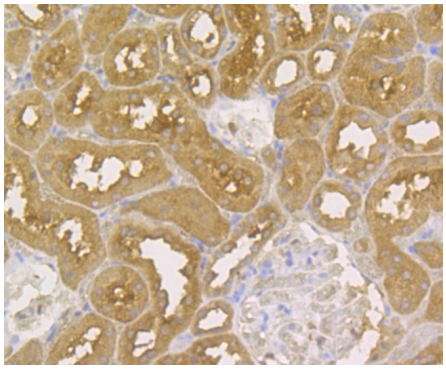

Fig5: Immunohistochemical analysis of paraffin-embedded rat kidney tissue using anti-GAPDH antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the antibody (R1210-1) at 1/100 dilution, for 30 minutes at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chrogen. Counter stained with hematoxylin and mounted with DPX.

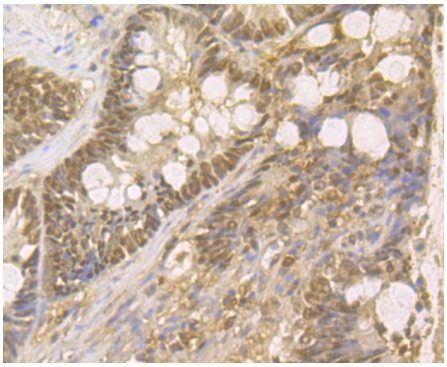

Fig6: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue using anti-GAPDH antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the antibody (R1210-1) at 1/100 dilution, for 30 minutes at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chrogen. Counter stained with hematoxylin and mounted with DPX.

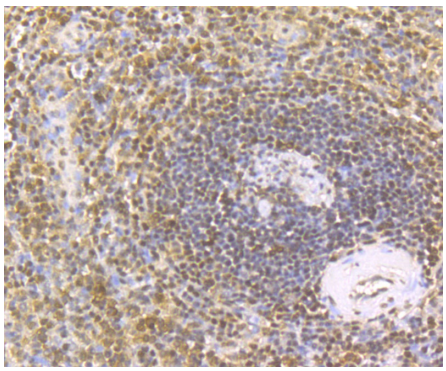

Fig7: Immunohistochemical analysis of paraffin-embedded human spleen tissue using anti-GAPDH antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the antibody (R1210-1) at 1/100 dilution, for 30 minutes at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chrogen. Counter stained with hematoxylin and mounted with DPX.

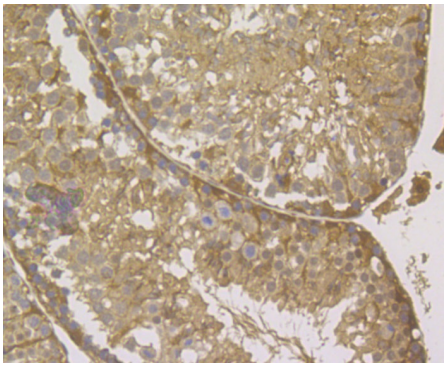

Fig8: Immunohistochemical analysis of paraffin-embedded mouse testis tissue using anti-GAPDH antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the antibody (R1210-1) at 1/100 dilution, for 30 minutes at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chrogen. Counter stained with hematoxylin and mounted with DPX.

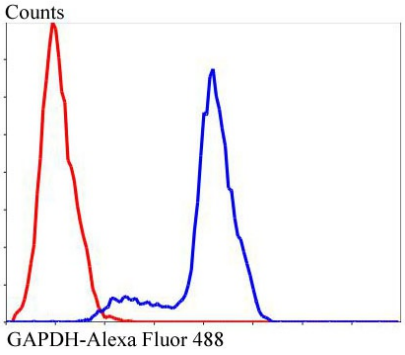

Fig9: Flow cytometric analysis of GAPDH was done on Hela cells. The cells were fixed, permeabilized and stained with GAPDH antibody at 1/100 dilution (blue) compared with an unlabelled control (cells without incubation with primary antibody; red). After incubation of the primary antibody on room temperature for an hour, the cells was stained with a Alexa Fluor™ 488-conjugated goat anti-rabbit IgG Secondary antibody at 1/500 dilution.

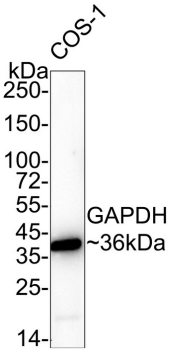

Fig10: Western blot analysis of GAPDH on COS-1 cell/tissue lysates with Rabbit anti-GAPDH antibody (R1210-1) at 1/20,000 dilution.

Lysates/proteins at 10 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 (R1210-1) at 1/20,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

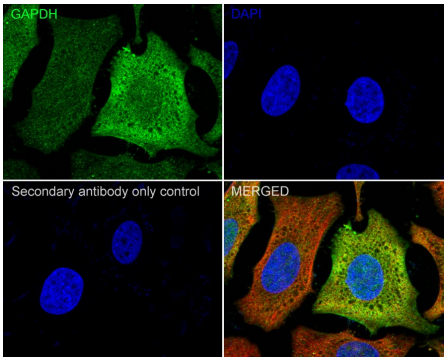

Fig11: Immunocytochemistry analysis of HeLa cells labeling GAPDH with Rabbit anti-GAPDH antibody (R1210-1) at 1/100 dilution.

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 (R1210-1) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. 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℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

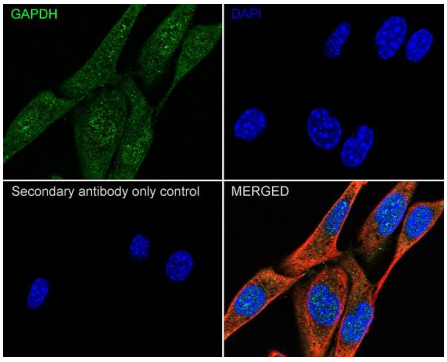

Fig12: Immunocytochemistry analysis of NIH/3T3 cells labeling GAPDH with Rabbit anti-GAPDH antibody (R1210-1) at 1/100 dilution.

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 (R1210-1) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. 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℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

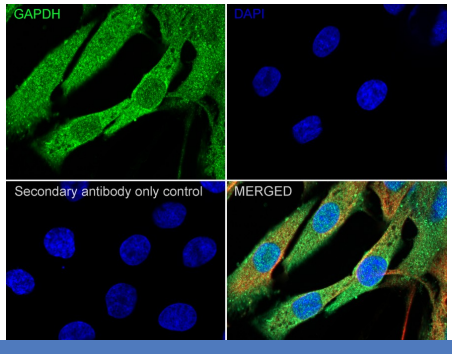

Fig13: Immunocytochemistry analysis of C6 cells labeling GAPDH with Rabbit anti-GAPDH antibody (R1210-1) at 1/100 dilution.

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 (R1210-1) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. 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.

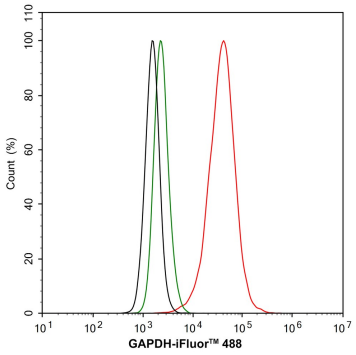

Fig14: Flow cytometric analysis of HeLa cells labeling GAPDH.

Cells were fixed and permeabilized. Then stained with the primary antibody (R1210-1, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ 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℃. 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. Allen R.W et al. Identification of the 37-kDa protein displaying a variable interaction with the erythroid cell membrane as glyceraldehyde-3-phosphate dehydrogenase. J Biol Chem 262:649-653 (1987).
2. Meyer-Siegler K et al. A human nuclear uracil DNA glycosylase is the 37-kDa subunit of glyceraldehyde-3-phosphate dehydrogenase. Proc Natl Acad Sci USA 88:8460-8464 (1991).