Anti-PHGDH Antibody [A10H9-R]

HA601349

Product Type:	Recombinant Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P
Molecular Wt:	Predicted band size: 57 kDa
Clone number:	A10H9-R

Description: 3-Phosphoglycerate dehydrogenase catalyzes the transition of 3-phosphoglycerate into 3-phosphohydroxypyruvate, which is the committed step in the phosphorylated pathway of L-serine biosynthesis. It is also essential in cysteine and glycine synthesis, which lie further downstream. This pathway represents the only way to synthesize serine in most organisms except plants, which uniquely possess multiple synthetic pathways. Nonetheless, the phosphorylated pathway that PHGDH participates in is still suspected to have an essential role in serine synthesis used in the developmental signaling of plants. Because of serine and glycine's role as neurotrophic factors in the developing brain, PHGDH has been shown to have high expression in glial and astrocyte cells during neural development.

Immunogen: Recombinant protein within human PHGDH aa 1-533.

Positive control: HeLa cell lysate, HEK-293 cell lysate, Jurkat cell lysate, MCF7 cell lysate, A549 cell lysate, HepG2 cell lysate, MDA-MB-468 cell lysate, SK-MEL-28 cell lysate, NIH/3T3 cell lysate, Neuro-2a cell lysate, RAW264.7 cell lysate, C6 cell lysate, mouse pancreas tissue lysate, rat pancreas tissue lysate, HeLa, NIH/3T3, human breast cancer tissue, human brain tissue, mouse brain tissue, rat brain tissue.

Subcellular location: Cytosol, extracellular exosome.

Database links: SwissProt: O43175 Human | Q61753 Mouse | O08651 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100
IHC-P	1:1,000

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% 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 A affinity purified.

Images

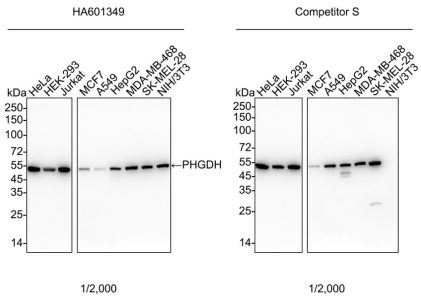

Fig1: Western blot analysis of PHGDH on different lysates with Mouse anti-PHGDH antibody (HA601349) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: HEK-293 cell lysate (20 µg/Lane)
Lane 3: Jurkat cell lysate (20 µg/Lane)
Lane 4: MCF7 cell lysate (20 µg/Lane)
Lane 5: A549 cell lysate (20 µg/Lane)
Lane 6: HepG2 cell lysate (20 µg/Lane)
Lane 7: MDA-MB-468 cell lysate (20 µg/Lane)
Lane 8: SK-MEL-28 cell lysate (20 µg/Lane)
Lane 9: NIH/3T3 cell lysate (20 µg/Lane)

Predicted band size: 57 kDa
Observed band size: 55 kDa

Exposure time: 2 minutes; ECL: K1801;

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 (HA601349) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution were 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.

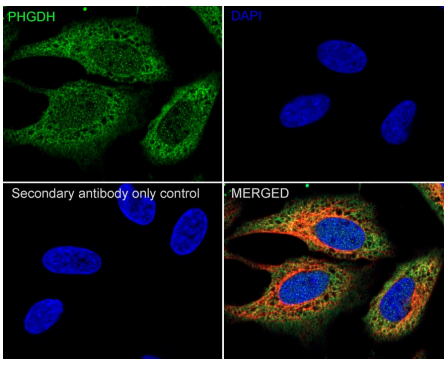

Fig2: Immunocytochemistry analysis of HeLa cells labeling PHGDH with Mouse anti-PHGDH antibody (HA601349) at 1/100 dilution.

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-PHGDH antibody (HA601349) 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) was used as the secondary antibody at 1/1,000 dilution.

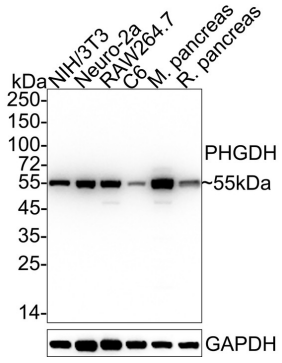

Fig3: Western blot analysis of PHGDH on different lysates with Mouse anti-PHGDH antibody (HA601349) at 1/2,000 dilution.

Lane 1: NIH/3T3 cell lysate (20 µg/Lane)
Lane 2: Neuro-2a cell lysate (20 µg/Lane)
Lane 3: RAW264.7 cell lysate (20 µg/Lane)
Lane 4: C6 cell lysate (20 µg/Lane)
Lane 5: Mouse pancreas tissue lysate (40 µg/Lane)
Lane 6: Rat pancreas tissue lysate (40 µg/Lane)

Predicted band size: 57 kDa
Observed band size: 55 kDa

Exposure time: 10 seconds; ECL: K1801;

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 (HA601349) 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.

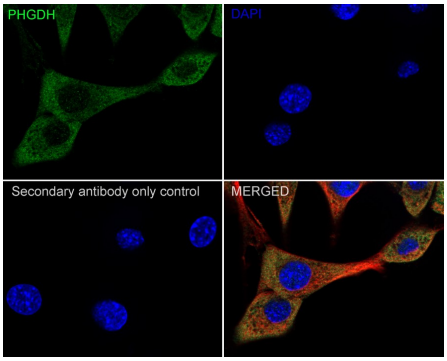

Fig4: Immunocytochemistry analysis of NIH/3T3 cells labeling PHGDH with Mouse anti-PHGDH antibody (HA601349) at 1/100 dilution.

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-PHGDH antibody (HA601349) 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) was used as the secondary antibody at 1/1,000 dilution.

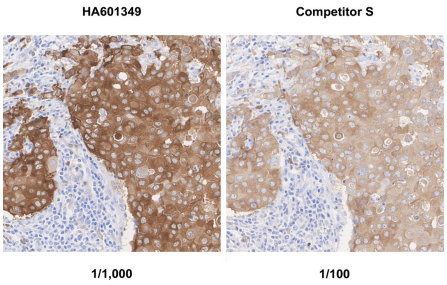

Fig5: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Mouse anti-PHGDH antibody (HA601349) at 1/1,000 dilution and competitor's antibody at 1/100 dilution.

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 (HA601349) at 1/100 dilution and competitor's antibody 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.

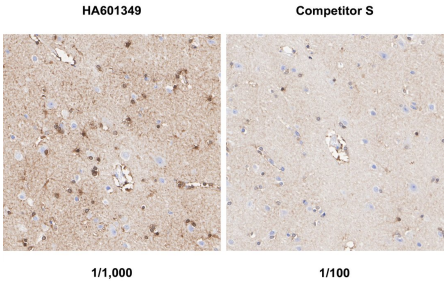

Fig6: Immunohistochemical analysis of paraffin-embedded human brain tissue with Mouse anti-PHGDH antibody (HA601349) at 1/1,000 dilution and competitor's antibody at 1/100 dilution.

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 (HA601349) at 1/100 dilution and competitor's antibody 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.

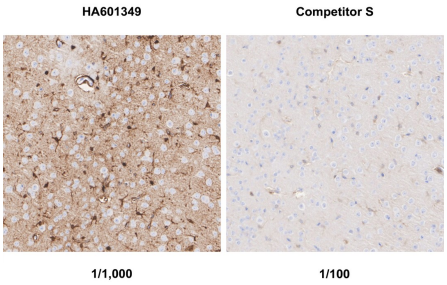

Fig7: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Mouse anti-PHGDH antibody (HA601349) at 1/1,000 dilution and competitor's antibody at 1/100 dilution.

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 (HA601349) at 1/100 dilution and competitor's antibody 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.

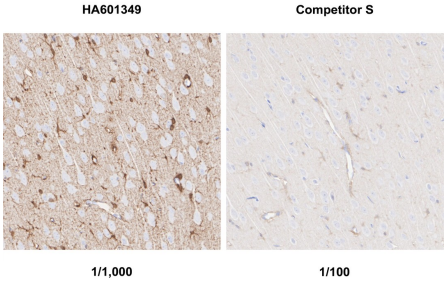

Fig8: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Mouse anti-PHGDH antibody (HA601349) at 1/1,000 dilution and competitor's antibody at 1/100 dilution.

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 (HA601349) at 1/100 dilution and competitor's antibody 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.

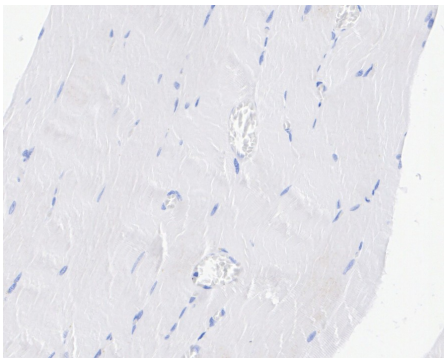

Fig9: Immunohistochemical analysis of paraffin-embedded human skeletal muscle tissue (negative) with Mouse anti-PHGDH antibody (HA601349) at 1/1,000 dilution.

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 (HA601349) 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. Rossi M et al. PHGDH heterogeneity potentiates cancer cell dissemination and metastasis. Nature. 2022 May
2. Shen L et al. PHGDH Inhibits Ferroptosis and Promotes Malignant Progression by Upregulating SLC7A11 in Bladder Cancer. Int J Biol Sci. 2022 Aug