

Anti-Glutaminase Antibody [SN68-09]

ET1611-5



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Rat, Mouse
Applications:	WB, IF-Cell, IF-Tissue, IHC-P
Molecular Wt:	Predicted band size: 74 kDa
Clone number:	SN68-09

Description: Glutamine is an important molecule involved in several cellular functions, including nitrogen and carbon transport, hepatic urea synthesis, renal ammoniogenesis, and gluconeogenesis. Glutamine is catabolized by either the liver-type (LGA) or kidney-type (KGA) glutaminase. KGA is mitochondrial specific protein whose expression in kidney is increased during metabolic acidosis. This process is mediated by an 8-base AU-sequence in KGA that functions as a pH-response element. The human KGA gene maps to chromosome 2, and produces three isoforms, designated KGA, GAC, and GAM, by alternative splicing. KGA is synthesized as a cytosolic protein that is transported to the mitochondria as an intermediate protein, and is further cleaved into the KGA isoform and the GAC isoform. The processing of the GAM isoform is unclear. The KGA isoform is abundant in brain and kidney, while the GAC isoform is principally expressed in cardiac muscle and pancreas. The GAM isoform is solely expressed in cardiac and skeletal muscle.

Immunogen: Synthetic peptide within human Glutaminase aa 50-100.

Positive control: 293T cell lysate, HeLa cell lysate, K-562 cell lysate, PC-12 cell lysate, rat brain tissue lysate, HeLa, HepG2, human tonsil tissue, human kidney tissue.

Subcellular location: Cytoplasm, Mitochondrion.

Database links: SwissProt: O94925 Human | D3Z7P3 Mouse | P13264 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:50-1:200

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4°C after thawing. Aliquot store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

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Orders:0086-571-88062880

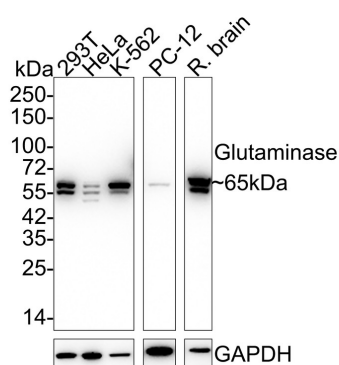
Technical:0086-571-89986345

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Images

Fig1: Western blot analysis of Glutaminase on different lysates with Rabbit anti-Glutaminase antibody (ET1611-5) at 1/1,000 dilution.



Lane 1: 293T cell lysate (20 µg/Lane)
 Lane 2: HeLa cell lysate (20 µg/Lane)
 Lane 3: K-562 cell lysate (20 µg/Lane)
 Lane 4: PC-12 cell lysate (20 µg/Lane)
 Lane 5: Rat brain tissue lysate (30 µg/Lane)

Predicted band size: 74 kDa

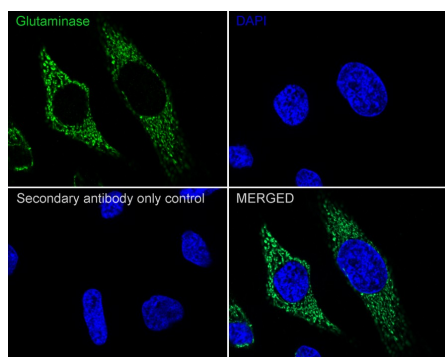
Observed band size: 65 kDa

Exposure time: 24 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 (ET1611-5) at 1/1,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig2: Immunocytochemistry analysis of HeLa cells labeling Glutaminase with Rabbit anti-Glutaminase antibody (ET1611-5) 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-Glutaminase antibody (ET1611-5) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. 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.

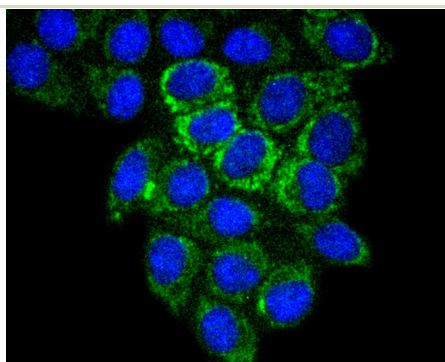


Fig3: ICC staining of Glutaminase in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1611-5, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

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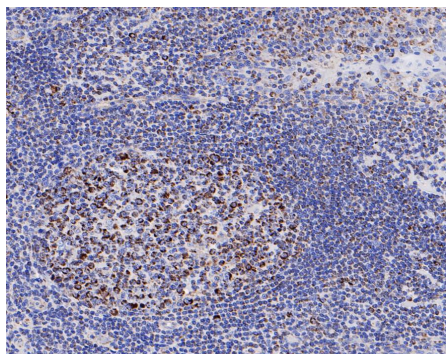


Fig4: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-Glutaminase antibody (ET1611-5) at 1/200 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (ET1611-5) at 1/200 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.

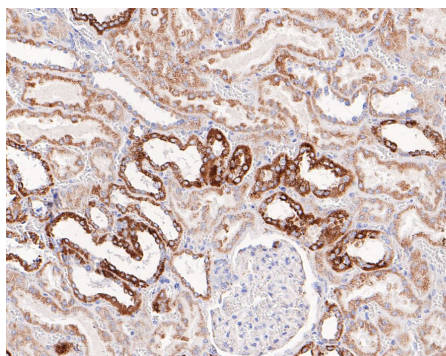


Fig5: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Glutaminase antibody (ET1611-5) at 1/200 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (ET1611-5) at 1/200 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.

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

Background References

1. Yu D et al. Kidney-type glutaminase (GLS1) is a biomarker for pathologic diagnosis and prognosis of hepatocellular carcinoma. *Oncotarget* 6:7619-31 (2015).
2. Gross MI et al. Antitumor activity of the glutaminase inhibitor CB-839 in triple-negative breast cancer. *Mol Cancer Ther* 13:890-901 (2014).

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