

Anti-GCN2 Antibody [JA03-83]

ET1704-68



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 187 kDa
Clone number:	JA03-83

Description: The family of stress-responsive protein kinases include HRI (heme-regulated inhibitor or EIF2AK1), PKR (EIF2AK2 or TIK), PERK (EIF2AK3) and GCN2 (EIF2AK4). These proteins phosphorylate the eukaryotic translation initiation factor 2 α (eIF2 α) on Ser 51 to regulate general and gene-specific protein synthesis. Phosphorylated eIF2 α acts as an inhibitor of its guanine nucleotide exchange factor eIF2B. GCN2, a unique eIF2 α kinase, exists in all eukaryotes from yeast to mammals. In mammals, expression of GCN2 is highest in liver and brain tissues. GCN2 primarily initiates the phosphorylation of eIF2 α in response to UV, but has been shown to increase phosphorylation activity in response to serum starvation. Also, substitution of Asp 83 for Ala on eIF2 α results in impaired phosphorylation by GCN2 and PKR, suggesting a contribution of remote residues to kinase-substrate recognition.

Immunogen: Synthetic peptide within Human GCN2 aa 1-50 / 1649.

Positive control: SKOV-3 cell lysates, A549, HeLa, MCF-7, human tonsil tissue, human breast carcinoma tissue, human placenta tissue.

Subcellular location: Cytoplasm.

Database links: SwissProt: Q9P2K8 Human

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

Storage Instruction: Shipped at 4°C. Store at +4°C short term (1-2 weeks). Store at -20°C long term.

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

Images

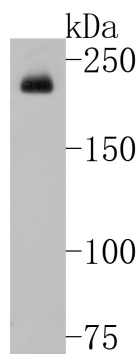


Fig1: Western blot analysis of GCN2 on SKOV-3 cell lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1704-68, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.

Fig2: Western blot analysis of GCN2 on different lysates with Rabbit anti-GCN2 antibody (ET1704-68) at 1/1,000 dilution.

Lane 1: A549-WT cell lysate

Lane 2: A549-KD GCN2 cell lysate

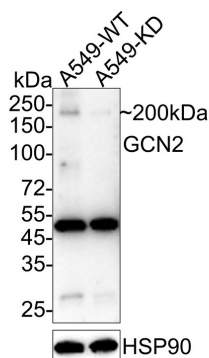
Lysates/proteins at 10 µg/Lane.

Predicted band size: 187 kDa

Observed band size: 200 kDa

Exposure time: 3 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 (ET1704-68) at 1/1,000 dilution was used in primary antibody diluent 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.

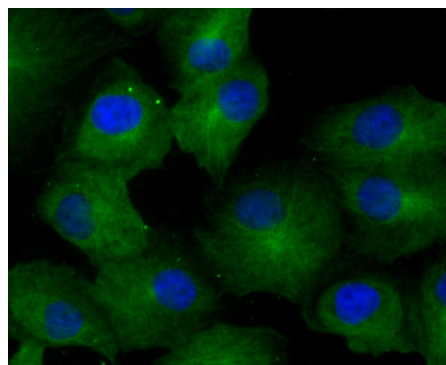


Fig3: ICC staining of GCN2 in A549 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1704-68, 1/50) for 1 hour at room 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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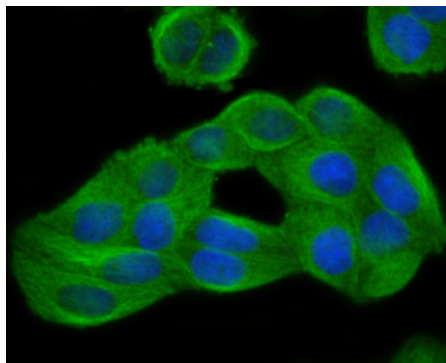


Fig4: ICC staining of GCN2 in HeLa cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1704-68, 1/50) for 1 hour at room 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).

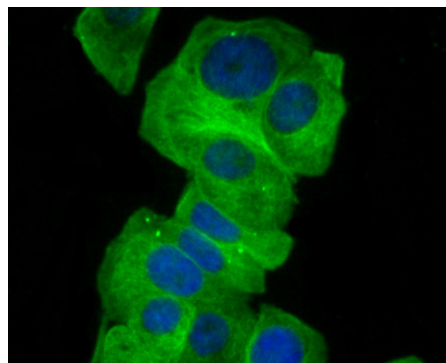


Fig5: ICC staining of GCN2 in MCF-7 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1704-68, 1/50) for 1 hour at room 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).

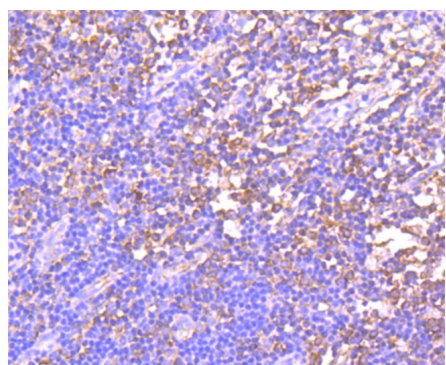


Fig6: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-GCN2 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1704-68, 1/50) for 30 minutes 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.

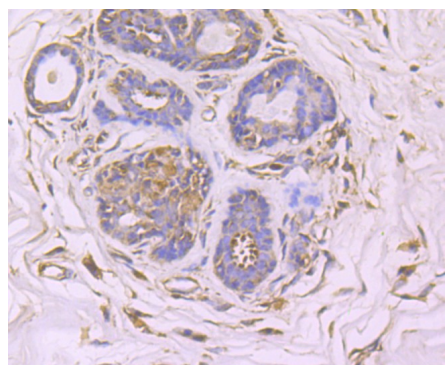


Fig7: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-GCN2 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1704-68, 1/50) for 30 minutes 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.

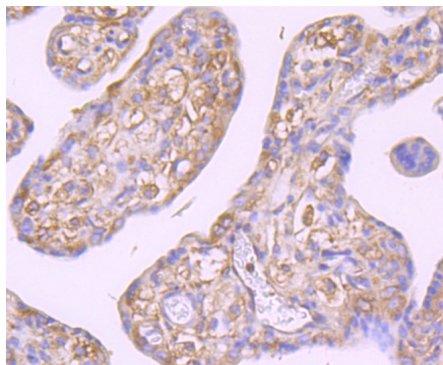


Fig8: Immunohistochemical analysis of paraffin-embedded human placenta tissue using anti-GCN2 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1704-68, 1/50) for 30 minutes 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.

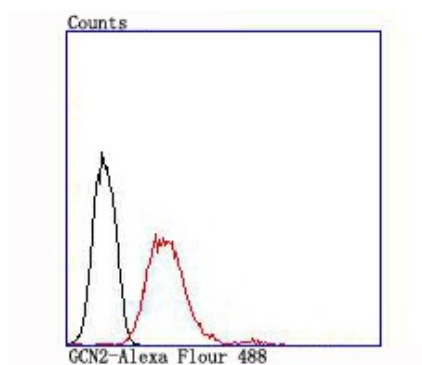


Fig9: Flow cytometric analysis of GCN2 was done on MCF-7 cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1704-68, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes. 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. Li C et al. Amino acid catabolism regulates hematopoietic stem cell proteostasis via a GCN2-eIF2 α axis. *Cell Stem Cell*. 2022 Jul
2. Missiaen R et al. GCN2 inhibition sensitizes arginine-deprived hepatocellular carcinoma cells to senolytic treatment. *Cell Metab*. 2022 Aug

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