Anti-eIF4E Antibody [JE60-70]

HA721192

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, FC
Molecular Wt:	Predicted band size: 25 kDa
Clone number:	JE60-70

Description: Eukaryotic translation initiation factor 4E, also known as eIF4E, is a protein that in humans is encoded by the EIF4E gene. Most eukaryotic cellular mRNAs are blocked at their 5'-ends with the 7-methyl-guanosine five-prime cap structure, m7GpppX (where X is any nucleotide). This structure is involved in several cellular processes including enhanced translational efficiency, splicing, mRNA stability, and RNA nuclear export. eIF4E is a eukaryotic translation initiation factor involved in directing ribosomes to the cap structure of mRNAs. It is a 24-kD polypeptide that exists as both a free form and as part of the eIF4F pre-initiation complex. Almost all cellular mRNA require eIF4E in order to be translated into protein. The eIF4E polypeptide is the rate-limiting component of the eukaryotic translation apparatus and is involved in the mRNA-ribosome binding step of eukaryotic protein synthesis. The other subunits of eIF4F are a 47-kD polypeptide, termed eIF4A,that possesses ATPase and RNA helicase activities, and a 220-kD scaffolding polypeptide, eIF4G. Some viruses cut eIF4G in such a way that the eIF4E binding site is removed and the virus is able to translate its proteins without eIF4E. Also some cellular proteins, the most notable being heat shock proteins, do not require eIF4E in order to be translated. Both viruses and cellular proteins achieve this through an internal ribosome entry site in the RNA.

Immunogen: Recombinant protein within Human eIF4E aa 118-217 / 217.

Positive control: HL-60 cell lysate, Daudi cell lysate, NIH/3T3 cell lysate, Mouse spleen tissue lysate, Rat spleen tissue lysate, human testis tissue, human colon carcinoma tissue, human breast carcinoma tissue, mouse hippocampus tissue, mouse brain tissue, SK-Br-3.

Subcellular location: Cytoplasm, P-body, Stress granule, Nucleus.

Database links: SwissProt: P06730 Human | P63073 Mouse | P63074 Rat

Recommended Dilutions:

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

Storage Buffer: 1*TBS (pH7.4), 0.05% 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

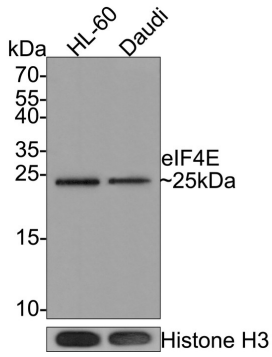

Fig1: Western blot analysis of eIF4E on different lysates with Rabbit anti-eIF4E antibody (HA721192) at 1/500 dilution.

Lane 1: HL-60 cell lysate
Lane 2: Daudi cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 25 kDa
Observed band size: 25 kDa

Exposure time: 2 minutes;

15% 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 (HA721192) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

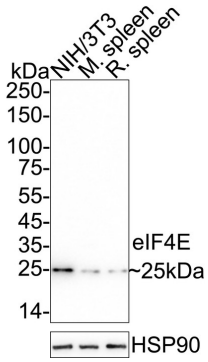

Fig2: Western blot analysis of eIF4E on different lysates with Rabbit anti-eIF4E antibody (HA721192) at 1/2,000 dilution.

Lane 1: NIH/3T3 cell lysate
Lane 2: Mouse spleen tissue lysate
Lane 3: Rat spleen tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 25 kDa
Observed band size: 25 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 (HA721192) at 1/2,000 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.

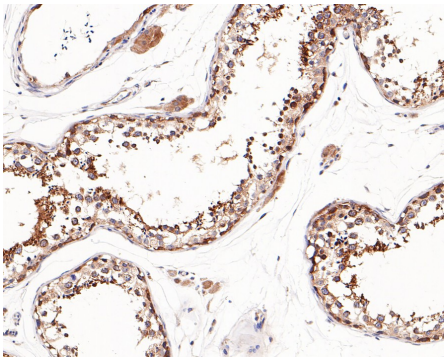

Fig3: Immunohistochemical analysis of paraffin-embedded human testis tissue with Rabbit anti-eIF4E antibody (HA721192) at 1/200 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (HA721192) 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.

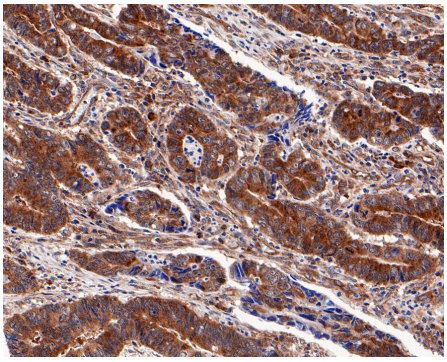

Fig4: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-eIF4E antibody (HA721192) at 1/200 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (HA721192) 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.

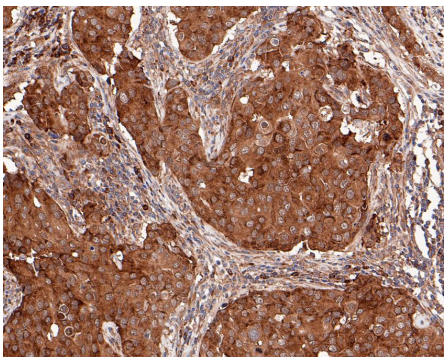

Fig5: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Rabbit anti-eIF4E antibody (HA721192) at 1/200 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (HA721192) 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.

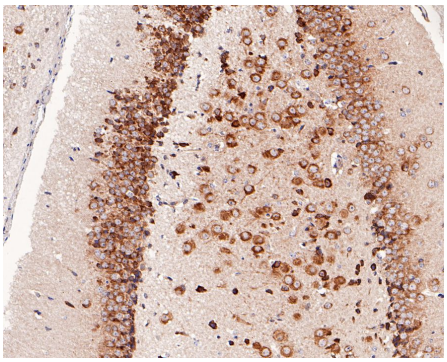

Fig6: Immunohistochemical analysis of paraffin-embedded mouse hippocampus tissue with Rabbit anti-eIF4E antibody (HA721192) at 1/200 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (HA721192) 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.

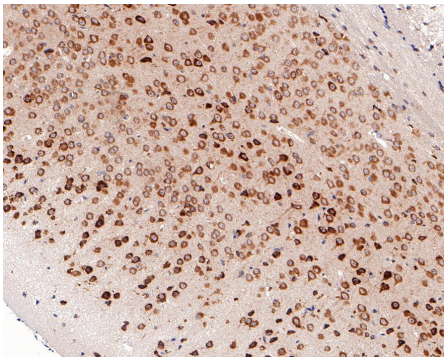

Fig7: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-eIF4E antibody (HA721192) at 1/200 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (HA721192) 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.

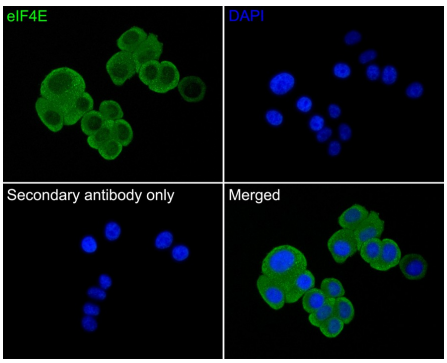

Fig8: Immunocytochemistry analysis of SK-Br-3 cells labeling eIF4E with Rabbit anti-eIF4E antibody (HA721192) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-eIF4E antibody (HA721192) at 1/50 dilution in 2% negative goat serum 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.

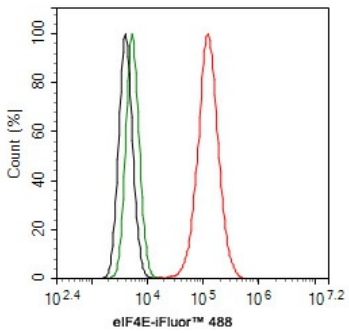

Fig9: Flow cytometric analysis of SK-Br-3 cells labeling eIF4E.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721192, 1ug/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. Ross-Kaschitza D et al. eIF4E and Interactors from Unicellular Eukaryotes. Int J Mol Sci. 2020 Mar
2. Christie M et al. eIF4E-homologous protein (4EHP): a multifarious cap-binding protein. FEBS J. 2021 Nov