Anti-Phospho-eIF4E (S209) Antibody [SU0396] ET1608-66

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, IP, FC
Molecular Wt:	Predicted band size: 25 kDa
Clone number:	SU0396

Description: Recognizes and binds the 7-methylguanosine-containing mRNA cap during an early step in the initiation of protein synthesis and facilitates ribosome binding by inducing the unwinding of the mRNAs secondary structures.In addition to its role in translation initiation, also acts as a regulator of translation and stability in the cytoplasm.Component of the CYFIP1-EIF4E-FMR1 complex which binds to the mRNA cap and mediates translational repression: in the complex, EIF4E mediates the binding to the mRNA cap.Component of a multiprotein complex that sequesters and represses translation of proneurogenic factors during neurogenesis.In P-bodies, component of a complex that mediates the storage of translationally inactive mRNAs in the cytoplasm and prevents their degradation.May play an important role in spermatogenesis through translational regulation of stage-specific mRNAs during germ cell development.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser209 of human eIF4E.

Positive control: HeLa cell lysate, HeLa treated with 50nM dexamethasone for 16 hours cell lysate, NIH/3T3 cell lysate, Mouse spleen tissue lysate, Rat spleen tissue lysate, NIH/3T3, human pancreas tissue, mouse brain tissue, mouse hippocampus tissue, rat spleen tissue, 293T.

Subcellular location: Cytoplasm, Nucleus.

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

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:50-1:200
IHC-P	1:50-1:200
IP	1-2μg/sample
FC	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

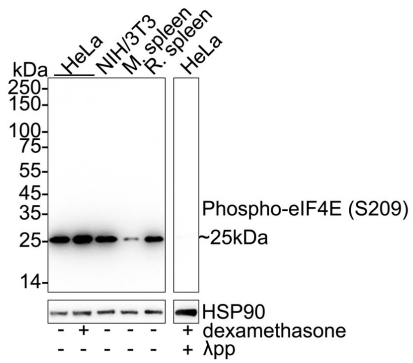

Fig1: Western blot analysis of Phospho-eIF4E (S209) on different lysates with Rabbit anti-Phospho-eIF4E (S209) antibody (ET1608-66) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 50nM dexamethasone for 16 hours cell lysate
Lane 3: NIH/3T3 cell lysate
Lane 4: Mouse spleen tissue lysate
Lane 5: Rat spleen tissue lysate
Lane 6: HeLa treated with 50nM dexamethasone for 16 hours cell lysate, then the membrane treated with λpp for 1 hour

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 42 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 (ET1608-66) 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.

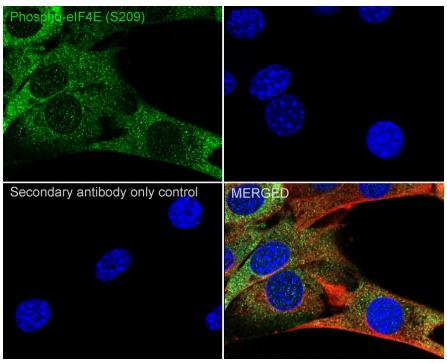

Fig2: Immunocytochemistry analysis of NIH/3T3 cells labeling Phospho-eIF4E (S209) with Rabbit anti-Phospho-eIF4E (S209) antibody (ET1608-66) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-Phospho-eIF4E (S209) antibody (ET1608-66) 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 (HA601187, 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.

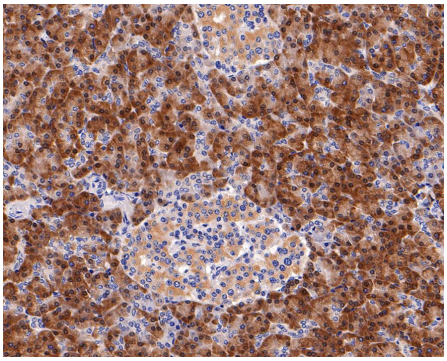

Fig3: Immunohistochemical analysis of paraffin-embedded human pancreas tissue with Rabbit anti-Phospho-eIF4E (S209) antibody (ET1608-66) at 1/200 dilution.

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 (ET1608-66) 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.

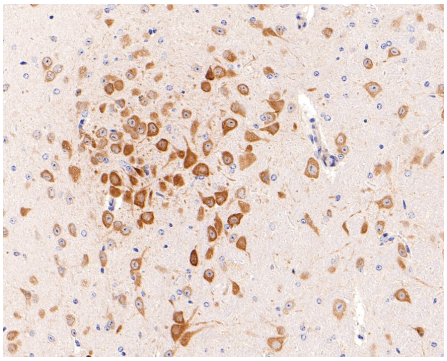

Fig4: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-Phospho-eIF4E (S209) antibody (ET1608-66) at 1/200 dilution.

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 (ET1608-66) 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.

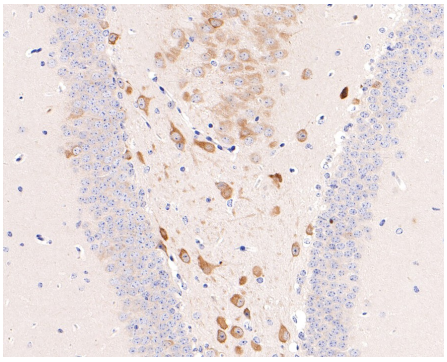

Fig5: Immunohistochemical analysis of paraffin-embedded mouse hippocampus tissue with Rabbit anti-Phospho-eIF4E (S209) antibody (ET1608-66) at 1/200 dilution.

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 (ET1608-66) 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.

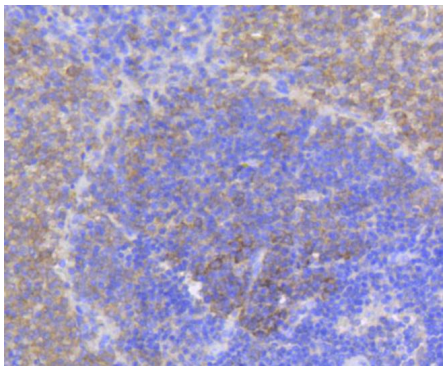

Fig6: Immunohistochemical analysis of paraffin-embedded rat spleen tissue using anti-Phospho-eIF4E (S209) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1608-66, 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.

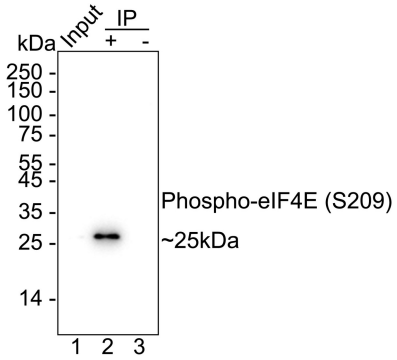

Fig7: Phospho-eIF4E (S209) was immunoprecipitated from 0.2 mg NIH/3T3 cell lysate with ET1608-66 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using ET1608-66 at 1/2,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: NIH/3T3 cell lysate (input)
Lane 2: ET1608-66 IP in NIH/3T3 cell lysate
Lane 3: Rabbit IgG instead of ET1608-66 in NIH/3T3 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 15 seconds; ECL: K1801

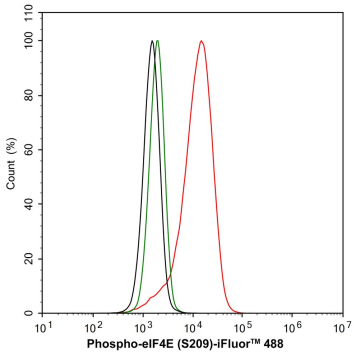

Fig8: Flow cytometric analysis of 293T cells labeling Phospho-eIF4E (S209).

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1608-66, 1/1,000) (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).

Background References

1. Zhang P et al. p53, MDM2, eIF4E and EGFR expression in nasopharyngeal carcinoma and their correlation with clinicopathological characteristics and prognosis: A retrospective study. Oncol Lett 9:113-118 (2015).
2. Zheng J et al. Phosphorylated Mnk1 and eIF4E are associated with lymph node metastasis and poor prognosis of nasopharyngeal carcinoma. PLoS One 9:e89220 (2014).