Anti-EIF2S1 Antibody

HA500385

Product Type:	Rabbit polyclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, FC, IF-Cell, IP
Molecular Wt:	Predicted band size: 36 kDa

Description: Functions in the early steps of protein synthesis by forming a ternary complex with GTP and initiator tRNA. This complex binds to a 40S ribosomal subunit, followed by mRNA binding to form a 43S pre-initiation complex. Junction of the 60S ribosomal subunit to form the 80S initiation complex is preceded by hydrolysis of the GTP bound to eIF-2 and release of an eIF-2-GDP binary complex. In order for eIF-2 to recycle and catalyze another round of initiation, the GDP bound to eIF-2 must exchange with GTP by way of a reaction catalyzed by eIF-2B. EIF2S1/eIF-2-alpha is a key component of the integrated stress response (ISR), required for adaptation to various stress: phosphorylation by metabolic-stress sensing protein kinases (EIF2AK1/HRI, EIF2AK2/PKR, EIF2AK3/PERK and EIF2AK4/GCN2) in response to stress converts EIF2S1/eIF-2-alpha in a global protein synthesis inhibitor, leading to an attenuation of cap-dependent translation, while concomitantly initiating the preferential translation of ISR-specific mRNAs, such as the transcriptional activators ATF4 and QRICH1, and hence allowing ATF4- and QRICH1-mediated reprogramming.

Immunogen: Recombinant protein within human EIF2S1 aa 116-315/315.

Positive control: HepG2 cell lysate, A431 cell lysate, Raji cell lysate, Hela cell lysate, PC-12 cell lysate, NIH/3T3 cell lysate, mouse colon tissue lysate, rat spleen tissue lysate, human kidney tissue, Hela, A375, NCI-H441.

Subcellular location: Cytoplasm, Stress granule, cytosol, Mitochondrion.

Database links: SwissProt: P05198 Human | Q6ZWX6 Mouse | P68101 Rat

Recommended Dilutions:

WB	1:500-1,000
IHC-P	1:1,900
FC	1:500-1:1,000
IF-Cell	1:100-1:200
IP	1-2μg/sample

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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

Purity: Immunogen affinity purified.

Images

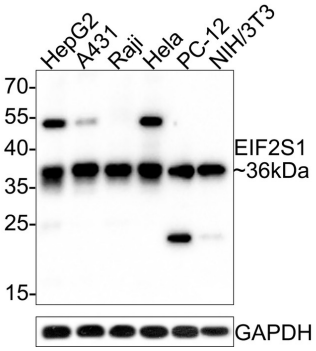

Fig1: Western blot analysis of EIF2S1 on different lysates with Rabbit anti-EIF2S1 antibody (HA500385) at 1/1,000 dilution.

Lane 1: HepG2 cell lysate
Lane 2: A431 cell lysate
Lane 3: Raji cell lysate
Lane 4: Hela cell lysate
Lane 5: PC-12 cell lysate
Lane 6: NIH/3T3 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 36 kDa
Observed band size: 36 kDa

Exposure time: 21 seconds;

12% 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 (HA500385) at 1/1,000 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.

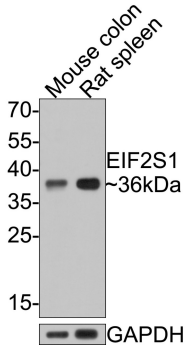

Fig2: Western blot analysis of EIF2S1 on different lysates with Rabbit anti-EIF2S1 antibody (HA500385) at 1/500 dilution.

Lane 1: Mouse colon tissue lysate
Lane 2: Rat spleen tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 36 kDa
Observed band size: 36 kDa

Exposure time: 30 seconds;

12% 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 (HA500385) 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.

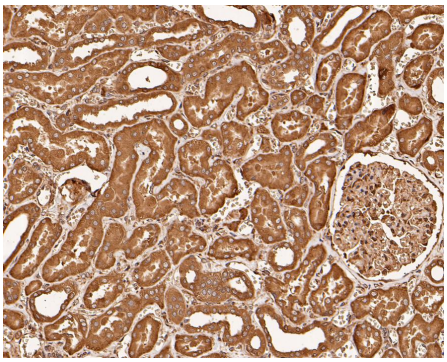

Fig3: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-EIF2S1 antibody (HA500385) at 1/1,900 dilution.

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 (HA500385) at 1/1,900 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.

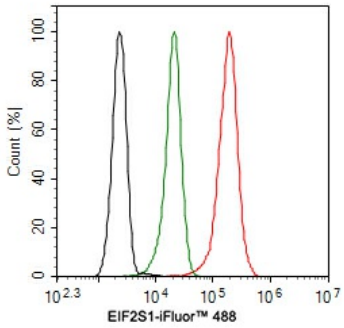

Fig4: Flow cytometric analysis of Hela cells labeling EIF2S1.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA500385, 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).

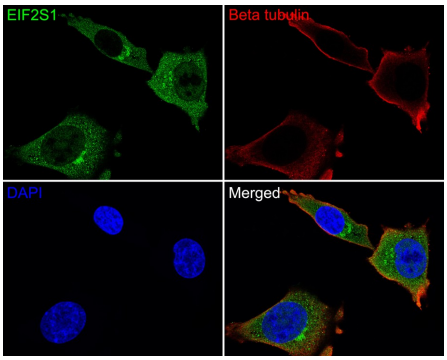

Fig5: Immunocytochemistry analysis of A375 cells labeling EIF2S1 with Rabbit anti-EIF2S1 antibody (HA500385) at 1/200 dilution.

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-EIF2S1 antibody (HA500385) at 1/200 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. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 647, HA1127) was used as the secondary antibody at 1/1,000 dilution.

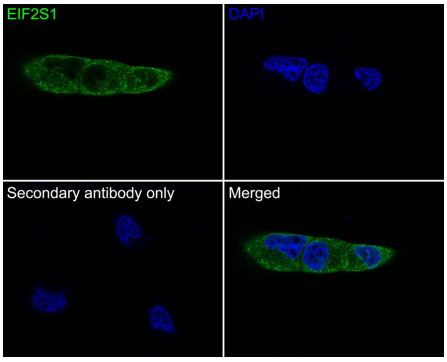

Fig6: Immunocytochemistry analysis of NCI-H441 cells labeling EIF2S1 with Rabbit anti-EIF2S1 antibody (HA500385) at 1/100 dilution.

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-EIF2S1 antibody (HA500385) at 1/100 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.

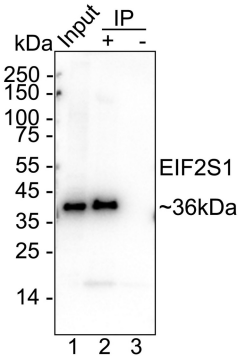

Fig7: EIF2S1 was immunoprecipitated from 0.2 mg HepG2 cell lysate with HA500385 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA500385 at 1/2,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HepG2 cell lysate (input)
Lane 2: HA500385 IP in HepG2 cell lysate
Lane 3: Rabbit IgG instead of HA500385 in HepG2 cell lysate

Blocking/Dilution buffer: primary antibody dilution (K1803)
Exposure time: 2 seconds; ECL: K1801

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

Background References

1. Li Y. et. al. LncRNAs LCETRL3 and LCETRL4 at chromosome 4q12 diminish EGFR-TKIs efficiency in NSCLC through stabilizing TDP43 and EIF2S1. Signal Transduct Target Ther. 2022 Jan
2. Schatz C. et. al. Dysregulation of Translation Factors EIF2S1, EIF5A and EIF6 in Intestinal-Type Adenocarcinoma (ITAC). Cancers (Basel). 2021 Nov