Anti-Phospho-EIF2S1 (S51) Antibody [SZ01-06] ET1603-14

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC
Molecular Wt:	Predicted band size: 36 kDa
Clone number:	SZ01-06

Description:	Phosphorylation of the eukaryotic initiation factor 2 (eIF2) α subunit is a well-documented mechanism to downregulate protein synthesis under a variety of stress conditions. Eukaryotic initiation factor 2 binds GTP and Met-tRNAi and transfers Met-tRNA to the 40S subunit to form the 43S preinitiation complex. eIF2 promotes a new round of translation initiation by exchanging GDP for GTP, a reaction catalyzed by eIF2B. Kinases that are activated by viral infection (PKR), endoplasmic reticulum stress (PERK/PEK), amino acid deprivation (GCN2), or heme deficiency (HRI) can phosphorylate the α subunit of eIF2. This phosphorylation stabilizes the eIF2-GDP-eIF2B complex and inhibits the turnover of eIF2B. Induction of PKR by IFN-γ and TNF-α induces potent phosphorylation of eIF2α at Ser51.
Immunogen:	Synthetic phospho-peptide corresponding to residues surrounding Ser51 of Human eIF-2a.
Positive control:	HeLa treated with 50nM Calyculin A for 3 hours whole cell lysate, NIH/3T3 treated with 100nM Calyculin A for 30 minutes whole cell lysate, C6 treated with 100nM Calyculin A for 30 minutes whole cell lysate, THP-1 cell lysate, C2C12 cell lysate, mouse spleen tissue, rat spleen tissue, human liver tissue, human pancreas tissue, mouse brain tissue, mouse placenta tissue, mouse pancreas tissue, human prostate carcinoma tissue, human breast carcinoma tissue, human colon carcinoma tissue, Hela.
Subcellular location:	Stress granule, Cytoplasm.
Database links:	SwissProt: P05198 Human | Q6ZWX6 Mouse | P68101 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:50-1:500
FC	1:50-1:100
IP	Use at an assay dependent concentration.

Storage Buffer:	1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

Images

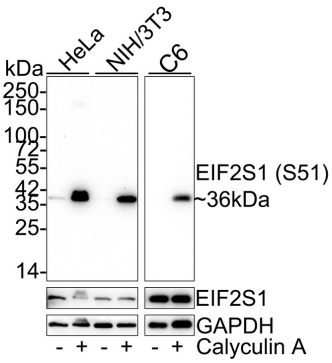

Fig1: Western blot analysis of Phospho-EIF2S1 (S51) on different lysates with Rabbit anti-Phospho-EIF2S1 (S51) antibody (ET1603-14) at 1/2,000 dilution.

Lane 1: HeLa whole cell lysate (15 µg/Lane)
Lane 2: HeLa treated with 50nM Calyculin A for 3 hours whole cell lysate (15 µg/Lane)
Lane 3: NIH/3T3 whole cell lysate (15 µg/Lane)
Lane 4: NIH/3T3 treated with 100nM Calyculin A for 30 minutes whole cell lysate (15 µg/Lane)
Lane 5: C6 whole cell lysate (20 µg/Lane)
Lane 6: C6 treated with 100nM Calyculin A for 30 minutes whole cell lysate (20 µg/Lane)

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

Exposure time: Lane 1-4: 2 minutes; Lane 5-6: 23 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 (ET1603-14) 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.

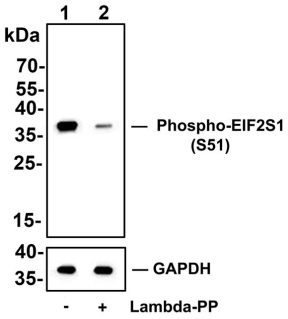

Fig2: Western blot analysis of Phospho-EIF2S1 (S51) on THP-1 cell lysates.

Lane 1: THP-1 cells, whole cell lysate, 10ug/lane
Lane 2: THP-1 cells treated with 2.8ug/ul lambda-PP for 30 minutes, whole cell lysates, 10ug/lane

All lanes :
Anti-Phospho-EIF2S1 (S51) antibody (ET1603-14) at 1/500 dilution. Anti-GAPDH antibody (ET1601-4) at 1/10,000 dilution. Goat Anti-Rabbit IgG H&L (HRP) (HA1001) at 1/200,000 dilution.

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

Blocking and diluting buffer: 5% BSA.

Exposure time: 3 minutes 43 seconds

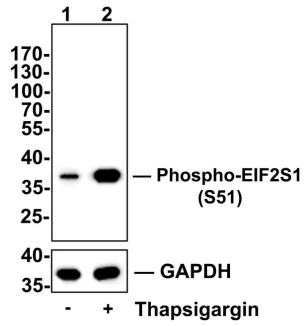

Fig3: Western blot analysis of Phospho-EIF2S1 (S51) on C2C12 cell lysates.

Lane 1: C2C12 cells, whole cell lysate, 10ug/lane
Lane 2: C2C12 cells treated with 300nM thapsigargin for 30 minutes, whole cell lysates, 10ug/lane

All lanes :
Anti-Phospho-EIF2S1 (S51) antibody (ET1603-14
) at 1:500 dilution. Anti-GAPDH antibody (ET1601-4) at 1:10,000 dilution. Goat Anti-Rabbit IgG H&L (HRP) (HA1001) at 1/200,000 dilution.

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

Blocking and diluting buffer: 5% BSA.

Exposure time: 3 minutes 43 seconds

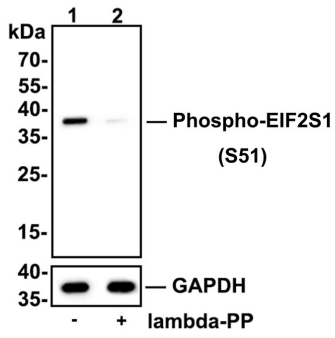

Fig4: Western blot analysis of Phospho-EIF2S1 (S51) on mouse spleen tissue lysates.

Lane 1: mouse spleen tissue, whole tissue lysate, 20ug/lane
Lane 2: mouse spleen tissue treated with 2.8ug/ul lambda-PP for 30 minutes, whole tissue lysates, 20ug/lane

All lanes :
Anti-Phospho-EIF2S1 (S51) antibody (ET1603-14) at 1/500 dilution. Anti-GAPDH antibody (ET1601-4) at 1/10,000 dilution. Goat Anti-Rabbit IgG H&L (HRP) (HA1001) at 1/200,000 dilution.

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

Blocking and diluting buffer: 5% BSA.

Exposure time: 3 minutes 43 seconds

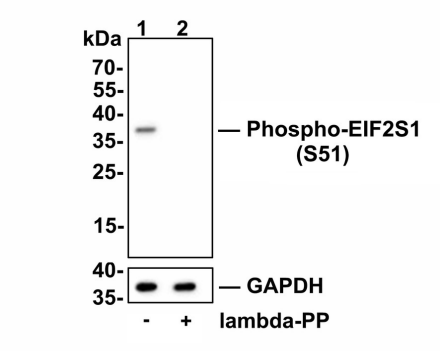

Fig5: Western blot analysis of Phospho-EIF2S1 (S51) on rat spleen tissue lysates.

Lane 1: rat spleen tissue, whole tissue lysate, 20ug/lane
Lane 2: rat spleen tissue treated with 2.8ug/ul lambda-PP for 30 minutes, whole tissue lysates, 20ug/lane

All lanes :
Anti-Phospho-EIF2S1 (S51) antibody (ET1603-14) at 1/500 dilution. Anti-GAPDH antibody (ET1601-4) at 1/10,000 dilution. Goat Anti-Rabbit IgG H&L (HRP) (HA1001) at 1/200,000 dilution.

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

Blocking and diluting buffer: 5% BSA.

Exposure time: 1 minute 15 seconds

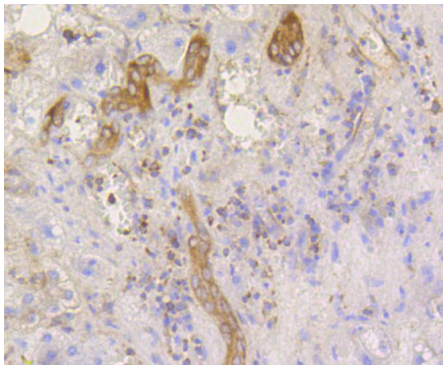

Fig6: Immunohistochemical analysis of paraffin-embedded human liver tissue using anti-Phospho-EIF2S1 (S51) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1603-14, 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.

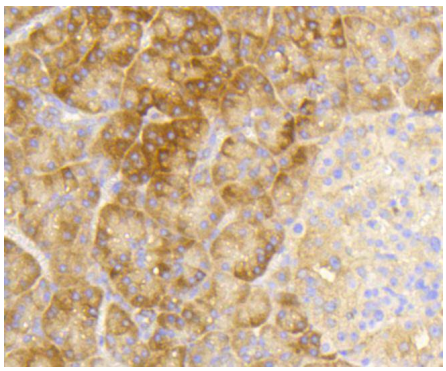

Fig7: Immunohistochemical analysis of paraffin-embedded human pancreas tissue using anti-Phospho-EIF2S1 (S51) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1603-14, 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.

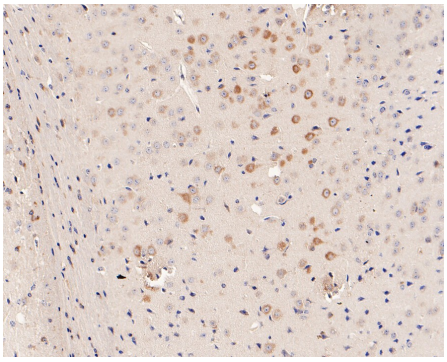

Fig8: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-Phospho-EIF2S1 (S51) antibody (ET1603-14) at 1/200 dilution.

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 (ET1603-14) 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.

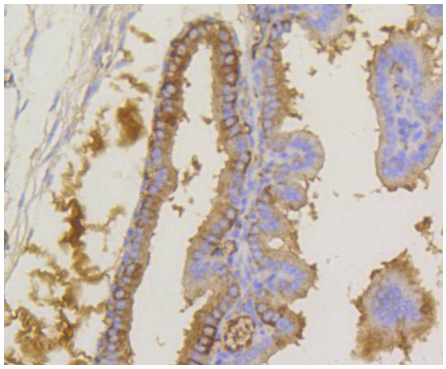

Fig9: Immunohistochemical analysis of paraffin-embedded mouse placenta tissue using anti-Phospho-EIF2S1 (S51) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1603-14, 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.

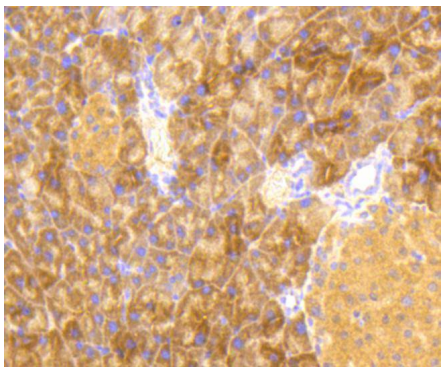

Fig10: Immunohistochemical analysis of paraffin-embedded mouse pancreas tissue using anti-Phospho-EIF2S1 (S51) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1603-14, 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.

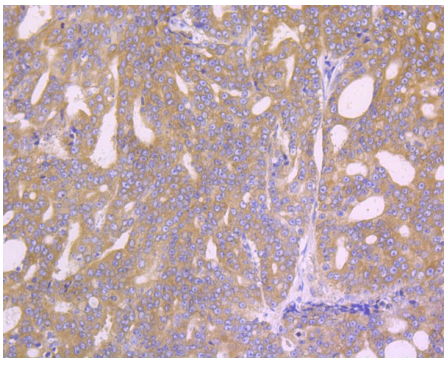

Fig11: Immunohistochemical analysis of paraffin-embedded human prostate carcinoma tissue using anti-Phospho-EIF2S1 (S51) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1603-14, 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.

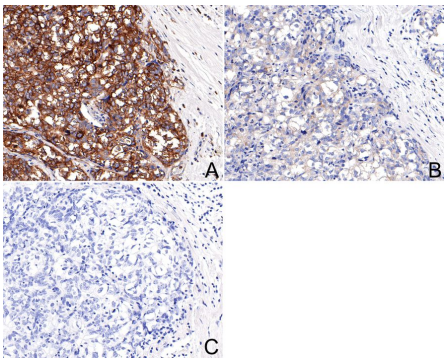

Fig12: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Rabbit anti-Phospho-EIF2S1 (S51) antibody (ET1603-14) at 1/200 dilution.

A: Untreated human breast carcinoma tissue
B: λ-PPase treated human breast carcinoma tissue
C: Negative control

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 (ET1603-14) 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.

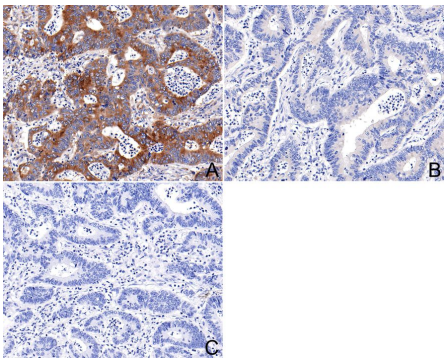

Fig13: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-Phospho-EIF2S1 (S51) antibody (ET1603-14) at 1/200 dilution.

A: Untreated human colon carcinoma tissue
B: λ-PPase treated human colon carcinoma tissue
C: Negative control

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 (ET1603-14) 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.

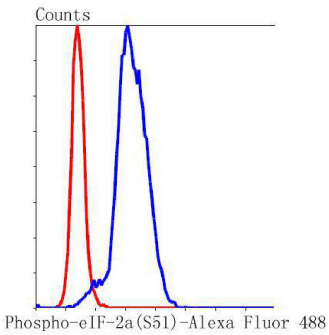

Fig14: Flow cytometric analysis of Phospho-EIF2S1 (S51) was done on Hela cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1603-14, 1/50) (blue). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated 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; red).

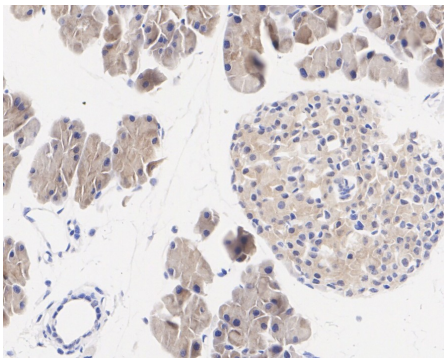

Fig15: Immunohistochemical analysis of paraffin-embedded mouse pancreas tissue with Rabbit anti-Phospho-EIF2S1 (S51) antibody (ET1603-14) at 1/1,000 dilution.

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 (ET1603-14) at 1/1,000 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.

Background References

1. Montalbano R et al. Endoplasmic reticulum stress plays a pivotal role in cell death mediated by the pan-deacetylase inhibitor panobinostat in human hepatocellular cancer cells. Transl Oncol 6:143-57 (2013).
2. Kanai R et al. Effect of 34.5 deletions on oncolytic herpes simplex virus activity in brain tumors. J Virol 86:4420-31 (2012).