Anti-eIF-6 Antibody [A6A3]

HA600051

Product Type:	Mouse monoclonal IgG2a, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, FC
Molecular Wt:	Predicted band size: 27 kDa
Clone number:	A6A3

Description: Binds to the 60S ribosomal subunit and prevents its association with the 40S ribosomal subunit to form the 80S initiation complex in the cytoplasm. Behaves as a stimulatory translation initiation factor downstream insulin/growth factors. Is also involved in ribosome biogenesis. Associates with pre-60S subunits in the nucleus and is involved in its nuclear export. Cytoplasmic release of TIF6 from 60S subunits and nuclear relocalization is promoted by a RACK1 (RACK1)-dependent protein kinase C activity. In tissues responsive to insulin, controls fatty acid synthesis and glycolysis by exerting translational control of adipogenic transcription factors such as CEBPB, CEBPD and ATF4 that have G/C rich or uORF in their 5'UTR. Required for ROS-dependent megakaryocyte maturation and platelets formation, controls the expression of mitochondrial respiratory chain genes involved in reactive oxygen species (ROS) synthesis.

Immunogen: Recombinant protein within human eIF-6 aa 1-200.

Positive control: HeLa cell lysate, HepG2 cell lysate, RAW264.7 cell lysate, NIH/3T3 cell lysate, mouse testis tissue lysate, rat kidney tissue lysate, rat testis tissue lysate, human colon cancer tissue, human kidney tissue, mouse kidney tissue, rat kidney tissue, HepG2, NIH/3T3.

Subcellular location: Cytoplasm, Nucleus, nucleolus.

Database links: SwissProt: P56537 Human | O55135 Mouse | Q3KRD8 Rat

Recommended Dilutions:

WB	1:1,000
IHC-P	1:1,000
IF-Cell	1:100
FC	1:1,000

Storage Buffer: PBS (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 G affinity purified.

Images

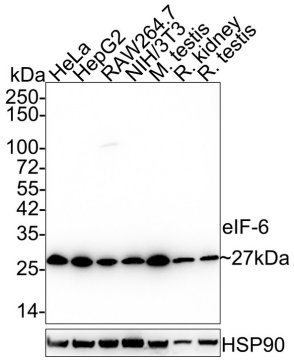

Fig1: Western blot analysis of eIF-6 on different lysates with Mouse anti-eIF-6 antibody (HA600051) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HepG2 cell lysate
Lane 3: RAW264.7 cell lysate
Lane 4: NIH/3T3 cell lysate
Lane 5: Mouse testis tissue lysate
Lane 6: Rat kidney tissue lysate
Lane 7: Rat testis tissue lysate

Lysates/proteins at 30 µg/Lane.

Predicted band size: 27 kDa
Observed band size: 27 kDa

Exposure time: 30 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 (HA600051) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Anti-Mouse IgG for IP Nano-secondary antibody (NBI02H) at 1/5,000 dilution was used for 1 hour at room temperature.

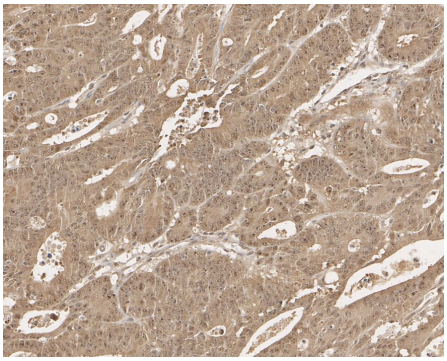

Fig2: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Mouse anti-eIF-6 antibody (HA600051) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (HA600051) 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.

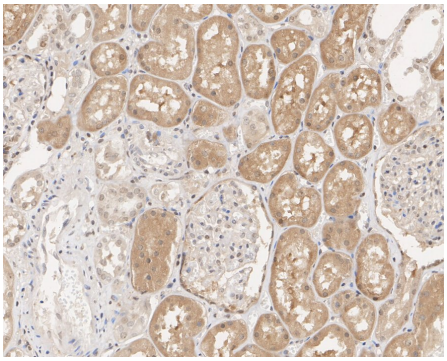

Fig3: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Mouse anti-eIF-6 antibody (HA600051) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (HA600051) 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.

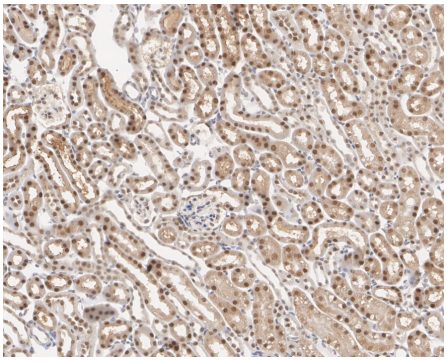

Fig4: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Mouse anti-eIF-6 antibody (HA600051) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (HA600051) 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.

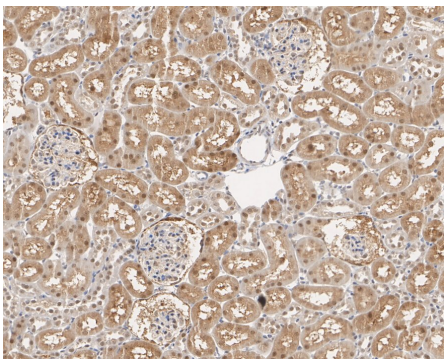

Fig5: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Mouse anti-eIF-6 antibody (HA600051) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (HA600051) 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.

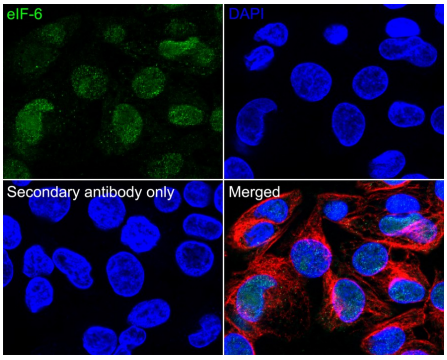

Fig6: Immunocytochemistry analysis of HepG2 cells labeling eIF-6 with Mouse anti-eIF-6 antibody (HA600051) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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 Mouse anti-eIF-6 antibody (HA600051) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) 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 (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

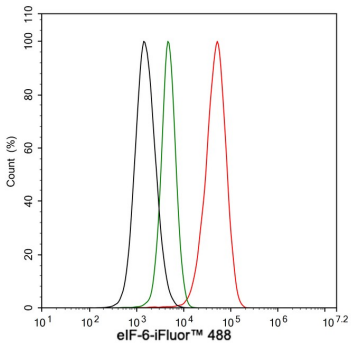

Fig7: Flow cytometric analysis of HepG2 cells labeling eIF-6.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA600051, 1μg/mL) (red) compared with Mouse IgG1 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-Mouse IgG Secondary antibody (HA1125) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

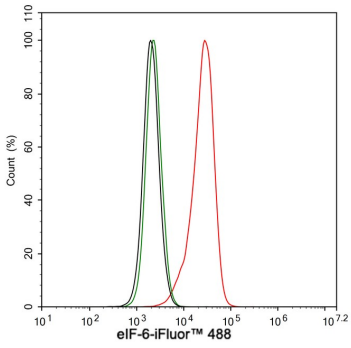

Fig8: Flow cytometric analysis of NIH/3T3 cells labeling eIF-6.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA600051, 1μg/mL) (red) compared with Mouse IgG1 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-Mouse IgG Secondary antibody (HA1125) 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. Liu Z. et. al. Long noncoding RNA PICSAR/miR-588/EIF6 axis regulates tumorigenesis of hepatocellular carcinoma by activating PI3K/AKT/mTOR signaling pathway. Cancer Sci. 2020 Nov
2. Pesce E. et. al. Discovery and Preliminary Characterization of Translational Modulators that Impair the Binding of eIF6 to 60S Ribosomal Subunits. Cells. 2020 Jan