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Anti-EIF6 Antibody Picoband™ (monoclonal, 2I11)

Catalog # ABO14828

Product Information

Application	WB, IHC, FC
Primary Accession	P56537
Host	Mouse
Isotype	Mouse IgG2a
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-EIF6 Antibody Picoband™ (monoclonal, 2I11) . Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500 □g/ml.

Additional Information

Gene ID	3692
Other Names	Eukaryotic translation initiation factor 6 {ECO:0000255|HAMAP-Rule:MF_03132}, eIF-6 {ECO:0000255|HAMAP-Rule:MF_03132}, B(2)GCN homolog, B4 integrin interactor, CAB, p27(BBP), EIF6 {ECO:0000255|HAMAP-Rule:MF_03132, ECO:0000312|HGNC:HGNC:6159}
Calculated MW	26599
Application Details	Western blot, 0.1-0.5 □g/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1 □g/ml Flow Cytometry, 1-3 □g/1x10^6 cells
Subcellular Localization	nucleolus. Cytoplasm
Tissue Specificity	Expressed at very high levels in colon carcinoma with lower levels in normal colon and ileum and lowest levels in kidney and muscle.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Clone Names	Clone: 2I11
Immunogen	E.coli-derived human EIF6 recombinant protein (Position: N66-T210). Human EIF6 shares 99.3% amino acid (aa) sequence identity with both mouse and rat EIF6.

Cross Reactivity	No cross-reactivity with other proteins.
Storage	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.

Protein Information

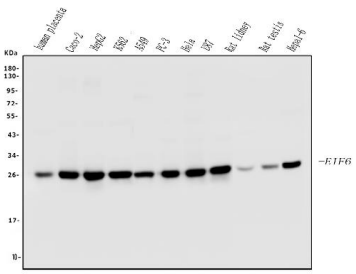
Name	EIF6 {ECO:0000255|HAMAP-Rule:MF_03132, ECO:0000312|HGNC:HGNC:6159}
Function	Binds to the 60S ribosomal subunit and prevents its association with the 40S ribosomal subunit to form the 80S initiation complex in the cytoplasm (PubMed:10085284, PubMed:14654845, PubMed:21536732, PubMed:32669547). Behaves 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 (PubMed:10085284, PubMed:14654845, PubMed:21536732). 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 (By similarity). Involved in miRNA-mediated gene silencing by the RNA-induced silencing complex (RISC). Required for both miRNA-mediated translational repression and miRNA-mediated cleavage of complementary mRNAs by RISC (PubMed:17507929). Modulates cell cycle progression and global translation of pre-B cells, its activation seems to be rate-limiting in tumorigenesis and tumor growth (By similarity).
Cellular Location	Cytoplasm. Nucleus, nucleolus. Note=Shuttles between cytoplasm and nucleus/nucleolus
Tissue Location	Expressed at very high levels in colon carcinoma with lower levels in normal colon and ileum and lowest levels in kidney and muscle (at protein level).

Background

EIF6 (Eukaryotic Translation Initiation Factor 6), also called EIF3A or ITGB4BP, is a human gene. By fluorescence in situ hybridization, Sanvito et al. (1998) mapped the ITGB4BP gene to 20q11.2. Ceci et al. (2003) demonstrated that the ribosomal 60S subunit is activated by release of EIF6. In the cytoplasm, EIF6 is bound to free 60S but not to 80S subunits. Furthermore, EIF6 interacts in the cytoplasm with RACK1, a receptor for activated protein kinase C. Gandin et al. (2008) demonstrated that mammalian eIF6 is required for efficient initiation of translation in vivo. Eif6-null mouse embryos were lethal at preimplantation. Heterozygous mice had 50% reduction of eIF6 levels in all tissues, and showed reduced mass of hepatic and adipose tissues due to a lower number of cells and to impaired G1/S cell cycle progression.

Images

Figure 1. Western blot analysis of EIF6 using anti-EIF6 antibody (M03581).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours.
The sample well of each lane was loaded with 50ug of



sample under reducing conditions.
Lane 1: human placenta tissue lysates,
Lane 2: human CACO-2 whole cell lysates,
Lane 3: human HEPG2 whole cell lysates,
Lane 4: human K562 whole cell lysates,
Lane 5: human A549 whole cell lysates,
Lane 6: human PC-3 whole cell lysates,
Lane 7: human Hela whole cell lysates,
Lane 8: human U87 whole cell lysates,
Lane 9: rat kidney tissue lysates,
Lane 10: rat testis tissue lysates,
Lane 11: mouse HEPA1-6 whole cell lysates.
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-EIF6 antigen affinity purified monoclonal antibody (Catalog # M03581) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for EIF6 at approximately 26KD. The expected band size for EIF6 is at 26KD.

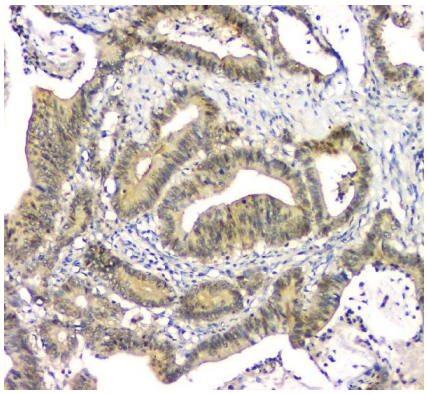


Figure 2. IHC analysis of EIF6 using anti-EIF6 antibody (M03581).
EIF6 was detected in paraffin-embedded section of human intestinal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-EIF6 Antibody (M03581) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

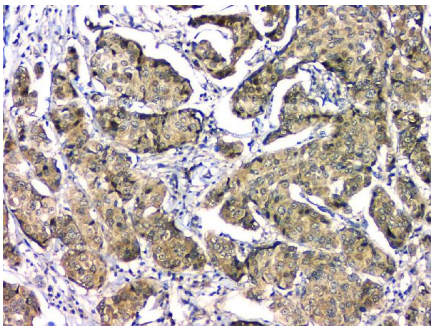
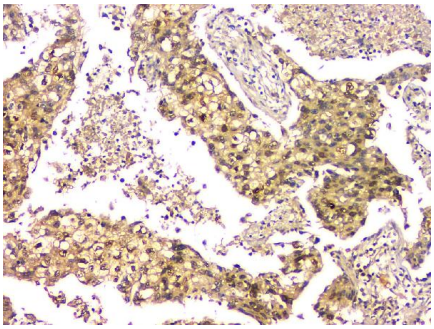


Figure 3. IHC analysis of EIF6 using anti-EIF6 antibody (M03581).
EIF6 was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-EIF6 Antibody (M03581) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

Figure 4. IHC analysis of EIF6 using anti-EIF6 antibody (M03581).
EIF6 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen



retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-EIF6 Antibody (M03581) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

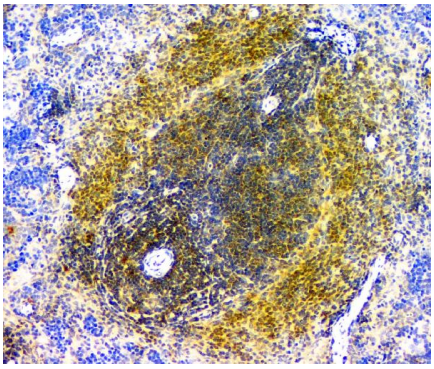


Figure 5. IHC analysis of EIF6 using anti-EIF6 antibody (M03581).
EIF6 was detected in paraffin-embedded section of rat spleen tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-EIF6 Antibody (M03581) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

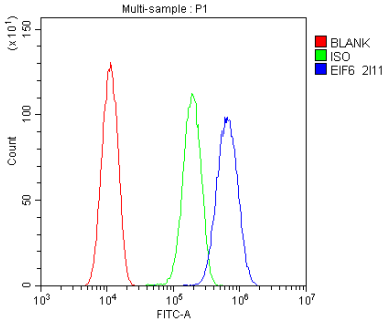


Figure 6. Flow Cytometry analysis of U20S cells using anti-EIF6 antibody (M03581).
Overlay histogram showing U20S cells stained with M03581 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-EIF6 Antibody (M03581, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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