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Anti-EIF5 Antibody Picoband™ (monoclonal, 2F13C4)

Catalog # ABO16232

Product Information

Application	WB, IF, ICC, FC
Primary Accession	P55010
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-EIF5 Antibody Picoband™ (monoclonal, 2F13C4) . Tested in Flow Cytometry, IF, ICC, WB applications. This antibody reacts with Human.
Reconstitution	Adding 0.2 ml of distilled water will yield a concentration of 500 □g/ml.

Additional Information

Gene ID	1983
Other Names	Eukaryotic translation initiation factor 5, eIF-5, EIF5
Calculated MW	49223
Application Details	Western blot, 0.25-0.5 □g/ml, Human Immunocytochemistry/Immunofluorescence, 5 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Source	Eukaryota
Contents	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.
Clone Names	Clone: 2F13C4
Immunogen	E.coli-derived human EIF5 recombinant protein (Position: D11-D423).
Purification	Immunogen affinity purified.
Storage	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 freezing and thawing.

Protein Information

Name	EIF5
Function	Component of the 43S pre-initiation complex (43S PIC), which binds to the

mRNA cap-proximal region, scans mRNA 5'-untranslated region, and locates the initiation codon (PubMed:11166181, PubMed:22813744, PubMed:24319994). In this complex, acts as a GTPase- activating protein, by promoting GTP hydrolysis by eIF2G (EIF2S3) (PubMed:11166181). During scanning, interacts with both EIF1 (via its C-terminal domain (CTD)) and EIF1A (via its NTD) (PubMed:22813744). This interaction with EIF1A contributes to the maintenance of EIF1 within the open 43S PIC (PubMed:24319994). When start codon is recognized, EIF5, via its NTD, induces eIF2G (EIF2S3) to hydrolyze the GTP (PubMed:11166181). Start codon recognition also induces a conformational change of the PIC to a closed state (PubMed:22813744). This change increases the affinity of EIF5-CTD for EIF2-beta (EIF2S2), which allows the release, by an indirect mechanism, of EIF1 from the PIC (PubMed:22813744). Finally, EIF5 stabilizes the PIC in its closed conformation (PubMed:22813744).

Cellular Location Cytoplasm.

Background

Eukaryotic translation initiation factor 5 is a protein that in humans is encoded by the EIF5 gene. Eukaryotic translation initiation factor-5 (EIF5) interacts with the 40S initiation complex to promote hydrolysis of bound GTP with concomitant joining of the 60S ribosomal subunit to the 40S initiation complex. The resulting functional 80S ribosomal initiation complex is then active in peptidyl transfer and chain elongations.

Images

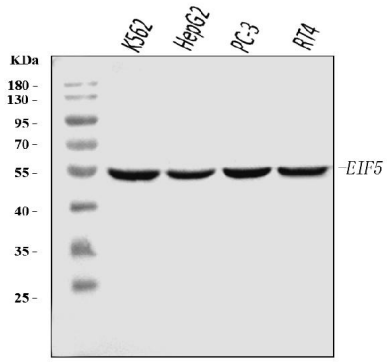
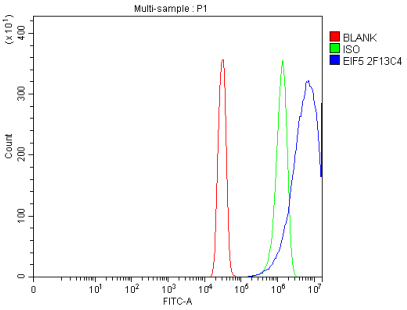


Figure 1. Western blot analysis of EIF5 using anti-EIF5 antibody (M04623).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human K562 whole cell lysates,
Lane 2: human HepG2 whole cell lysates,
Lane 3: human PC-3 whole cell lysates,
Lane 4: human RT4 whole cell lysates.
After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA 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-EIF5 antigen affinity purified monoclonal antibody (Catalog # M04623) 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 EIF5 at approximately 49 kDa. The expected band size for EIF5 is at 49 kDa.

Figure 2. Flow Cytometry analysis of SiHa cells using anti-EIF5 antibody (M04623).
Overlay histogram showing SiHa cells stained with M04623 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-EIF5 Antibody (M04623, 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.

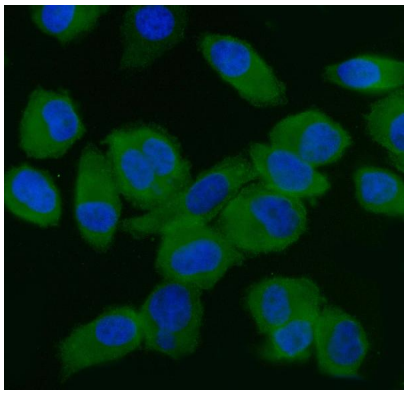


Figure 3. IF analysis of EIF5 using anti-EIF5 antibody (M04623).
EIF5 was detected in an immunocytochemical section of PC-3 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-EIF5 Antibody (M04623) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.