

# Anti-EEF2 Picoband<sup>™</sup> Antibody (monoclonal, 5F5)

Catalog # ABO14989

Specification

## Anti-EEF2 Picoband<sup>™</sup> Antibody (monoclonal, 5F5) - Product Information

Application Primary Accession Host Isotype Reactivity Clonality Format **Description** Anti-EEF2 Picoband™ WB, IHC, IF, ICC, FC <u>P13639</u> Mouse Mouse IgG1 Rat, Human, Mouse Monoclonal Lyophilized

Anti-EEF2 Picoband<sup>™</sup> Antibody (monoclonal, 5F5) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.

**Reconstitution** Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

#### Anti-EEF2 Picoband<sup>™</sup> Antibody (monoclonal, 5F5) - Additional Information

Gene ID 1938

**Other Names** Elongation factor 2, EF-2, 3.6.5.-, EEF2, EF2

Calculated MW 95 kDa KDa

**Application Details** Western blot, 0.25-0.5 μg/ml, Human, Mouse, Rat<br> Immunohistochemistry (Paraffin-embedded Section), 2-5 μg/ml, Huma<br> Immunocytochemistry/Immunofluorescence, 5 μg/ml, Human<br> Flow Cytometry, 1-3 μg/1x10^6 cells, Human, Mouse, Rat<br>

Contents Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.

Immunogen

A synthetic peptide corresponding to a sequence at the N-terminus of human EEF2/Elongation factor 2, identical to the related mouse and rat sequences.

**Purification** Immunogen affinity purified.

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.



## Anti-EEF2 Picoband<sup>™</sup> Antibody (monoclonal, 5F5) - Protein Information

Name EEF2

Synonyms EF2

Function

Catalyzes the GTP-dependent ribosomal translocation step during translation elongation (PubMed:<a href="http://www.uniprot.org/citations/26593721" target="\_blank">26593721</a>). During this step, the ribosome changes from the pre-translocational (PRE) to the posttranslocational (POST) state as the newly formed A-site-bound peptidyl- tRNA and P-site-bound deacylated tRNA move to the P and E sites, respectively (PubMed:<a href="http://www.uniprot.org/citations/26593721" target="\_blank">26593721</a>). Catalyzes the coordinated movement of the two tRNA molecules, the mRNA and conformational changes in the ribosome (PubMed:<a href="http://www.uniprot.org/citations/26593721" target="\_blank">26593721" target="\_blank">26593721</a>).

**Cellular Location** Cytoplasm. Nucleus. Note=Phosphorylation by CSK promotes cleavage and SUMOvlation-dependent nuclear translocation of the C- terminal cleavage product.

#### Anti-EEF2 Picoband<sup>™</sup> Antibody (monoclonal, 5F5) - Protocols

Provided below are standard protocols that you may find useful for product applications.

- <u>Western Blot</u>
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- <u>Cell Culture</u>

Anti-EEF2 Picoband<sup>™</sup> Antibody (monoclonal, 5F5) - Images

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Figure 1. Western blot analysis of EEF2 using anti-EEF2 antibody (M00830-2). 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 Hela whole cell lysates,
- Lane 2: human HEPG2 whole cell lysates,
- Lane 3: human Jurkat whole cell lysates,
- Lane 4: human U20S whole cell lysates,
- Lane 5: rat PC-12 whole cell lysates,
- Lane 6: mouse NIH/3T3 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-EEF2 antigen affinity purified monoclonal antibody (Catalog # M00830-2) at 0.5  $\mu$ g/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 EEF2 at approximately 95KD. The expected band size for EEF2 is at 95KD.



Figure 2. IHC analysis of EEF2 using anti-EEF2 antibody (M00830-2).

EEF2 was detected in paraffin-embedded section of human breast 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 2  $\mu$ g/ml mouse anti-EEF2 Antibody (M00830-2) 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 EEF2 using anti-EEF2 antibody (M00830-2).

EEF2 was detected in paraffin-embedded section of human rectal 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 2  $\mu$ g/ml mouse anti-EEF2 Antibody (M00830-2) 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 EEF2 using anti-EEF2 antibody (M00830-2).

EEF2 was detected in paraffin-embedded section of human liver 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 2  $\mu$ g/ml mouse anti-EEF2 Antibody (M00830-2) 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 EEF2 using anti-EEF2 antibody (M00830-2).

EEF2 was detected in paraffin-embedded section of human rectal 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 2  $\mu$ g/ml mouse anti-EEF2 Antibody (M00830-2) 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. IF analysis of EEF2 using anti-EEF2 antibody (M00830-2).

EEF2 was detected in immunocytochemical section of HEPG2 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  $\mu$ g/mL mouse anti-EEF2 Antibody (M00830-2) 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.



Figure 7. Flow Cytometry analysis of HEPA1-6 cells using anti-EEF2 antibody (M00830-2).

Overlay histogram showing HEPA1-6 cells stained with M00830-2 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-EEF2 Antibody (M00830-2, 1  $\mu$ g/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10  $\mu$ g/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1  $\mu$ g/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample (Red line) was also used as a control.





Figure 8. Flow Cytometry analysis of HL-60 cells using anti-EEF2 antibody (M00830-2). Overlay histogram showing HL-60 cells stained with M00830-2 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-EEF2 Antibody (M00830-2, 1  $\mu$ g/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10  $\mu$ g/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1  $\mu$ g/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample (Red line) was also used as a control.



Figure 9. Flow Cytometry analysis of NRK cells using anti-EEF2 antibody (M00830-2). Overlay histogram showing NRK cells stained with M00830-2 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-EEF2 Antibody (M00830-2, 1  $\mu$ g/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10  $\mu$ g/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1  $\mu$ g/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

### Anti-EEF2 Picoband<sup>™</sup> Antibody (monoclonal, 5F5) - Background

Eukaryotic elongation factor 2is aproteinthat in humans is encoded by theEEF2gene. This gene encodes a member of the GTP-binding translation elongation factor family. This protein is an essential factor for protein synthesis. It promotes the GTP-dependent translocation of the nascent protein chain from the A-site to the P-site of the ribosome. This protein is completely inactivated by EF-2 kinase phosporylation.