

# Anti-eRF1/ETF1 Antibody Picoband™ (monoclonal, 3E5)

Catalog # ABO14987

### Specification

# Anti-eRF1/ETF1 Antibody Picoband™ (monoclonal, 3E5) - Product Information

Application Primary Accession Host Isotype Reactivity Clonality Format Description WB, IHC, IF, ICC, FC <u>P62495</u> Mouse Mouse IgG2b Rat, Human, Mouse Monoclonal Lyophilized

Anti-eRF1/ETF1 Antibody Picoband<sup>™</sup> (monoclonal, 3E5) . 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-eRF1/ETF1 Antibody Picoband<sup>™</sup> (monoclonal, 3E5) - Additional Information

Gene ID 2107

**Other Names** 

Eukaryotic peptide chain release factor subunit 1, Eukaryotic release factor 1, eRF1, Protein Cl1, TB3-1, ETF1, ERF1, RF1, SUP45L1

Calculated MW 49 kDa KDa

**Application Details** Western blot, 0.25-0.5 μg/ml, Human, Mouse, Rat<br> Immunohistochemistry (Paraffin-embedded Section), 2-5 μg/ml, Human, Rat<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 E.coli-derived human eRF1/ETF1 recombinant protein (Position: D9-K342).

**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-eRF1/ETF1 Antibody Picoband<sup>™</sup> (monoclonal, 3E5) - Protein Information

Name ETF1

Synonyms ERF1, RF1, SUP45L1

#### Function

Component of the eRF1-eRF3-GTP ternary complex, a ternary complex that mediates translation termination in response to the termination codons (PubMed:<a href="http://www.uniprot.org/citations/10676813" target=" blank">10676813</a>, PubMed:<a href="http://www.uniprot.org/citations/16777602" target=" blank">16777602</a>, PubMed:<a href="http://www.uniprot.org/citations/24486019" target=" blank">24486019</a>, PubMed:<a href="http://www.uniprot.org/citations/26245381" target="\_blank">26245381</a>, PubMed:<a href="http://www.uniprot.org/citations/27863242" target="\_blank">27863242</a>, PubMed:<a href="http://www.uniprot.org/citations/36638793" target=" blank">36638793</a>, PubMed:<a href="http://www.uniprot.org/citations/7990965" target=" blank">7990965</a>). The eRF1-eRF3-GTP complex binds to a stop codon in the ribosomal A-site (PubMed:<a href="http://www.uniprot.org/citations/26245381" target=" blank">26245381</a>, PubMed:<a href="http://www.uniprot.org/citations/27863242" target=" blank">27863242</a>, PubMed:<a href="http://www.uniprot.org/citations/36638793" target="\_blank">36638793</a>). ETF1/ERF1 is responsible for stop codon recognition and inducing hydrolysis of peptidyl-tRNA (PubMed:<a href="http://www.uniprot.org/citations/26245381" target=" blank">26245381</a>, PubMed:<a href="http://www.uniprot.org/citations/27863242" target=" blank">27863242</a>, PubMed:<a href="http://www.uniprot.org/citations/36638793" target=" blank">36638793</a>). Following GTP hydrolysis, eRF3 (GSPT1/ERF3A or GSPT2/ERF3B) dissociates, permitting ETF1/eRF1 to accommodate fully in the A-site and mediate hydrolysis of peptidyl-tRNA (PubMed:<a href="http://www.uniprot.org/citations/10676813" target=" blank">10676813</a>, PubMed:<a href="http://www.uniprot.org/citations/16777602" target="\_blank">16777602</a>, PubMed:<a href="http://www.uniprot.org/citations/26245381" target=" blank">26245381</a>, PubMed:<a href="http://www.uniprot.org/citations/27863242" target="blank">27863242</a>). Component of the transient SURF complex which recruits UPF1 to stalled ribosomes in the context of nonsense-mediated decay (NMD) of mRNAs containing premature stop codons (PubMed:<a href="http://www.uniprot.org/citations/19417104" target=" blank">19417104</a>). Required for SHFL-mediated translation termination which inhibits programmed ribosomal frameshifting (-1PRF) of mRNA from viruses and cellular genes (PubMed:<a href="http://www.uniprot.org/citations/30682371" target="\_blank">30682371</a>).

Cellular Location Cytoplasm.

# Anti-eRF1/ETF1 Antibody Picoband<sup>™</sup> (monoclonal, 3E5) - Protocols

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

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

# Anti-eRF1/ETF1 Antibody Picoband<sup>™</sup> (monoclonal, 3E5) - Images





Figure 1. Western blot analysis of eRF1/ETF1 using anti-eRF1/ETF1 antibody (M04157-1). 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 Jurkat whole cell lysates,

Lane 3: human K562 whole cell lysates,

Lane 4: human Raji whole cell lysates,

Lane 5: human HEPG2 whole cell lysates,

Lane 6: human HEPG2 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-eRF1/ETF1 antigen affinity purified monoclonal antibody (Catalog # M04157-1) 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 eRF1/ETF1 at approximately 49KD. The expected band size for eRF1/ETF1 is at 49KD.



Figure 2. Western blot analysis of eRF1/ETF1 using anti-eRF1/ETF1 antibody (M04157-1). 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: rat liver tissue lysates,



- Lane 2: rat testis tissue lysates,
- Lane 3: rat kidney tissue lysates,
- Lane 4: rat PC-12 whole cell lysates,
- Lane 5: mouse liver tissue lysates,
- Lane 6: mouse testis tissue lysates,
- Lane 7: mouse kidney tissue lysates,
- Lane 8: mouse RAW264.7 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-eRF1/ETF1 antigen affinity purified monoclonal antibody (Catalog # M04157-1) 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 eRF1/ETF1 at approximately 49KD. The expected band size for eRF1/ETF1 is at 49KD.



Figure 3. IHC analysis of eRF1/ETF1 using anti-eRF1/ETF1 antibody (M04157-1).

eRF1/ETF1 was detected in paraffin-embedded section of human tonsil 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-eRF1/ETF1 Antibody (M04157-1) 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 eRF1/ETF1 using anti-eRF1/ETF1 antibody (M04157-1).



eRF1/ETF1 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-eRF1/ETF1 Antibody (M04157-1) overnight at 4°C. Biotinylated goat anti-mouse lgG 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 eRF1/ETF1 using anti-eRF1/ETF1 antibody (M04157-1).

eRF1/ETF1 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-eRF1/ETF1 Antibody (M04157-1) overnight at 4°C. Biotinylated goat anti-mouse lgG 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. IHC analysis of eRF1/ETF1 using anti-eRF1/ETF1 antibody (M04157-1).

eRF1/ETF1 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 2  $\mu$ g/ml mouse anti-eRF1/ETF1 Antibody (M04157-1) overnight at 4°C. Biotinylated goat anti-mouse lgG 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 7. IHC analysis of eRF1/ETF1 using anti-eRF1/ETF1 antibody (M04157-1).

eRF1/ETF1 was detected in paraffin-embedded section of rat pancreas 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-eRF1/ETF1 Antibody (M04157-1) 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 8. IF analysis of eRF1/ETF1 using anti-eRF1/ETF1 antibody (M04157-1).

eRF1/ETF1 was detected in immunocytochemical section of A431 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-eRF1/ETF1 Antibody (M04157-1) 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 9. Flow Cytometry analysis of Caco-2 cells using anti-eRF1/ETF1 antibody (M04157-1). Overlay histogram showing Caco-2 cells stained with M04157-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-eRF1/ETF1 Antibody (M04157-1, 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 10. Flow Cytometry analysis of HEPA1-6 cells using anti-eRF1/ETF1 antibody (M04157-1). Overlay histogram showing HEPA1-6 cells stained with M04157-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-eRF1/ETF1 Antibody (M04157-1, 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 11. Flow Cytometry analysis of RH35 cells using anti-eRF1/ETF1 antibody (M04157-1). Overlay histogram showing RH35 cells stained with M04157-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-eRF1/ETF1 Antibody



(M04157-1, 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-eRF1/ETF1 Antibody Picoband<sup>™</sup> (monoclonal, 3E5) - Background

Eukaryotic translation termination factor 1 (eRF1), also known asTB3-1, is a protein that in humans is encoded by the ETF1 gene. It is mapped to 5q31.2. This gene encodes a class-1 polypeptide chain release factor. The encoded protein plays an essential role in directing termination of mRNA translation from the termination codons UAA, UAG and UGA. This protein is a component of the SURF complex which promotes degradation of prematurely terminated mRNAs via the mechanism of nonsense-mediated mRNA decay (NMD). Alternate splicing results in multiple transcript variants. Pseudogenes of this gene are found on chromosomes 6, 7, and X.