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

Catalog # ABO14987

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P62495
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-eRF1/ETF1 Antibody Picoband™ (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.

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	49031
Application Details	Western blot, 0.25-0.5 □g/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5 □g/ml, Human, Rat Immunocytochemistry/Immunofluorescence, 5 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human, Mouse, Rat
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Clone Names	Clone: 3 E5
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.

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:10676813, PubMed:16777602, PubMed:24486019, PubMed:26245381, PubMed:27863242, PubMed:36638793, PubMed:7990965). The eRF1-eRF3-GTP complex binds to a stop codon in the ribosomal A-site (PubMed:26245381, PubMed:27863242, PubMed:36638793). ETF1/ERF1 is responsible for stop codon recognition and inducing hydrolysis of peptidyl-tRNA (PubMed:26245381, PubMed:27863242, PubMed:36638793). 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:10676813, PubMed:16777602, PubMed:26245381, PubMed:27863242). 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:19417104). Required for SHFL-mediated translation termination which inhibits programmed ribosomal frameshifting (-1PRF) of mRNA from viruses and cellular genes (PubMed:30682371).
Cellular Location	Cytoplasm.

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.

Images

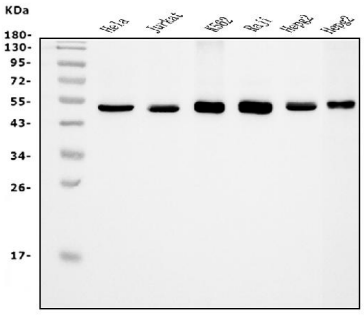


Figure 1. Western blot analysis of eRF1/ETF1 using anti-eRF1/ETF1 antibody (M04157-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours.
The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human 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 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 eRF1/ETF1 at approximately 49KD. The expected band size for eRF1/ETF1 is at 49KD.

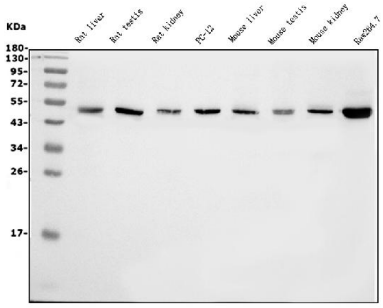


Figure 2. Western blot analysis of eRF1/ETF1 using anti-eRF1/ETF1 antibody (M04157-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: rat liver 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 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 eRF1/ETF1 at approximately 49KD. The expected band size for eRF1/ETF1 is at 49KD.

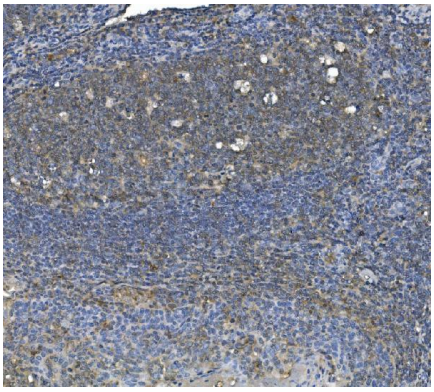
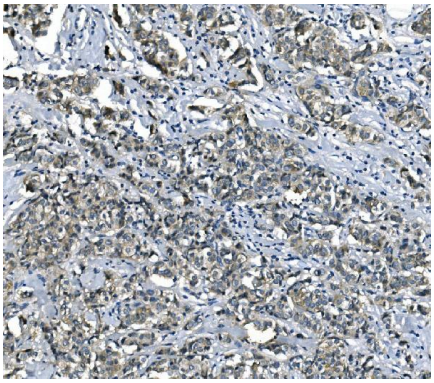


Figure 3. IHC analysis of eRF1/ETF1 using anti-eRF1/ETF1 antibody (M04157-1).
eRF1/ETF1 was detected in paraffin-embedded section of human tonsil tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/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 µg/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.

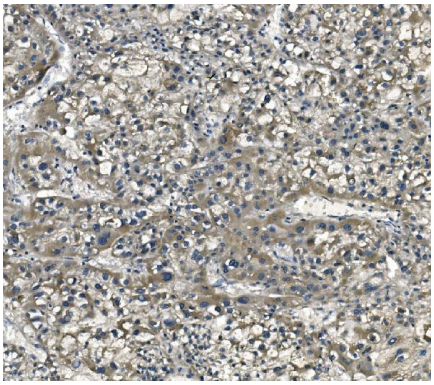


Figure 5. IHC analysis of eRF1/ETF1 using anti-eRF1/ETF1 antibody (M04157-1).
eRF1/ETF1 was detected in paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/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.

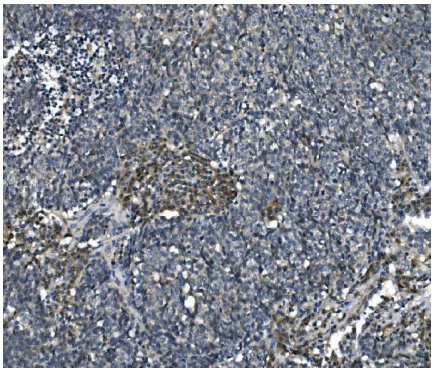


Figure 6. IHC analysis of eRF1/ETF1 using anti-eRF1/ETF1 antibody (M04157-1).
eRF1/ETF1 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/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.

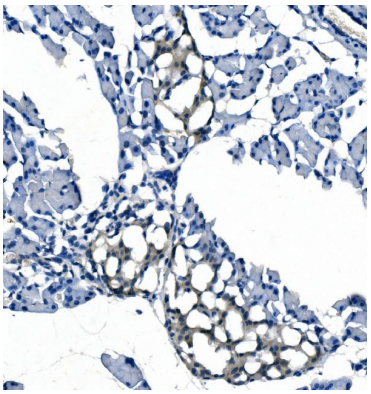
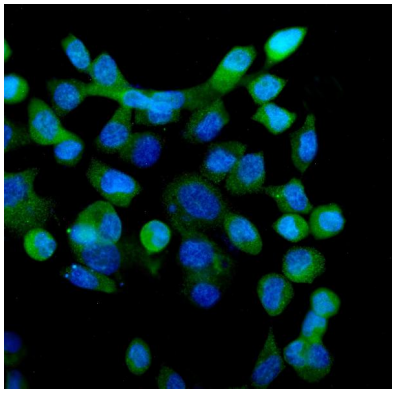


Figure 7. IHC analysis of eRF1/ETF1 using anti-eRF1/ETF1 antibody (M04157-1).
eRF1/ETF1 was detected in paraffin-embedded section of rat pancreas tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/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.

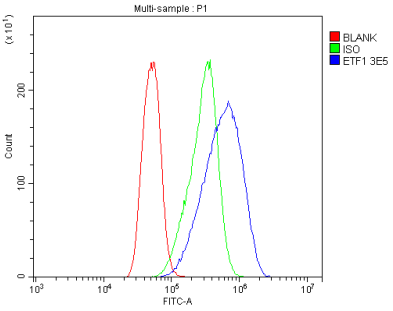


Figure 9. Flow Cytometry analysis of Caco-2 cells using anti-eRF1/ETF1 antibody (M04157-1).
Overlay histogram showing Caco-2 cells stained with M04157-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-eRF1/ETF1 Antibody (M04157-1, 1 µg/1x10^6 cells) 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.

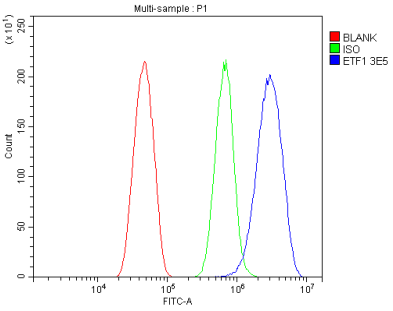


Figure 10. Flow Cytometry analysis of HEPA1-6 cells using anti-eRF1/ETF1 antibody (M04157-1).
Overlay histogram showing HEPA1-6 cells stained with M04157-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-eRF1/ETF1 Antibody (M04157-1, 1 µg/1x10^6 cells) 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.

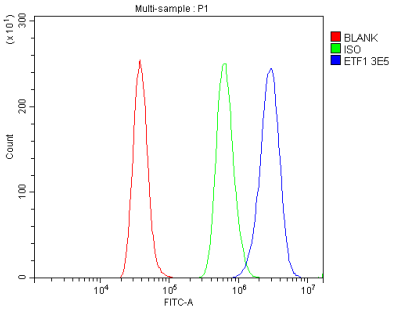


Figure 11. Flow Cytometry analysis of RH35 cells using anti-eRF1/ETF1 antibody (M04157-1).
Overlay histogram showing RH35 cells stained with M04157-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-eRF1/ETF1 Antibody (M04157-1, 1 µg/1x10^6 cells) for 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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