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Anti-mtTFA/TFAM Antibody Picoband®

Catalog Number: PB9447

About TFAM

TFAM (Transcription factor A, mitochondrial), also known as TCF6 or TCF6L2, is a 162-amino acid protein that activates transcription of each mitochondrial DNA (mtDNA) strand by binding to an element of approximately 30 nucleotides present in both the light-strand and the heavy-strand promoters. By Southern blot analysis of restriction enzyme digests of human/Chinese hamster somatic cell hybrid lines, Milatovich et al. (1992) mapped TFAM sequences, which they called MTTF1, to 3 different chromosomes: chromosomes 10, 7p, and 11q. By PCR-based screening of a somatic cell hybrid panel and by fluorescence in situ hybridization, Scott (2007) stated that the sequences mapped to chromosomes 7p (TCF6L1) and 11q (MTTF1, or TCF6L3) are pseudogenes. Larsson et al. (1997) mapped the mouse mitochondrial transcription factor A gene (Tfam) to the central part of mouse chromosome 10. This region exhibits syntenic homology with human 10q21. Mitochondrial transcription factor A is a key activator of mitochondrial transcription in mammals. It also has a role in mitochondrial DNA replication, since transcription generates an RNA primer necessary for initiation of mtDNA replication.

Overview

Product Name	Anti-mtTFA/TFAM Antibody Picoband®
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-mtTFA/TFAM Antibody Picoband® catalog # PB9447. Tested in IP, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	IP, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Storage Instructions	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.
Host	Rabbit
Uniprot ID	Q00059

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence at the N-terminus of human mtTFA, different from the related mouse and rat sequences by five amino acids.
Cross Reactivity	No cross-reactivity with other proteins
Isotype	Rabbit IgG

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Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.1-0.5ug/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Human Immunocytochemistry/Immunofluorescence, 5ug/ml, Human Immunoprecipitation, 0.5-2 ug/ml, Human

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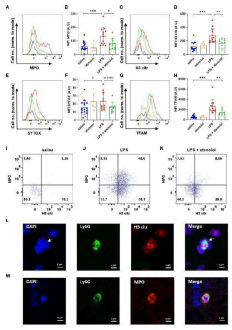
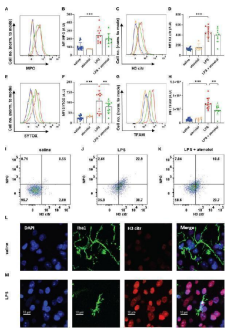
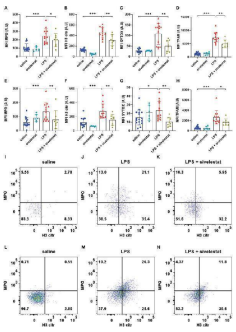
Anti-mtTFA/TFAM Antibody Picoband® (PB9447) Images



Atenolol decreased the production of NETs induced by LPS. Fluorescence intensity for a representative animal of each group for the extracellular trap markers MPO (A) , H3 citr (C) , Sytox (E) and TFAM (G) in saline (blue), atenolol (orange), LPS (red) and LPS + atenolol (green). Quantification of mean fluorescence intensities (MFIs) in live neutrophils for the extracellular trap markers MPO (B) , H3 citr (D) , Sytox (F) and TFAM (H) for saline (blue), atenolol (orange), LPS (red) and LPS + atenolol (green) groups. Representative dot plot of living neutrophils co-expressing MPO and H3 citr in saline (I) , LPS (J) and LPS + atenolol (K) groups. Representative pictures of Ly6G+ neutrophils (green) labeling with citrullinated histone H3 (H3 citr) (red). The nucleus of cells was labeled with DAPI (in blue). Arrow shows a neutrophil producing an ET (L) . Representative pictures of Ly6G+ neutrophils (green) labeling with myeloperoxidase (MPO) (red). The nucleus of cells was labeled with DAPI (in blue) (M) . Saline n=16, atenolol n=6, LPS n=13, LPS + atenolol n=10. * p



Atenolol decreased the production of MiETs induced by LPS. Fluorescence intensity for a representative animal of each group for the extracellular trap markers MPO (A) , H3 citr (C) , Sytox (E) and TFAM (G) in saline (blue), atenolol (orange), LPS (red) and LPS + atenolol (green) groups. Quantification of mean fluorescence intensities (MFIs) in live microglia for the extracellular traps markers MPO (B) , H3 citr (D) , Sytox (F) and TFAM (H) for saline (blue), atenolol (orange), LPS (red) and LPS + atenolol (green) groups. Representative dot plot of living microglia co-expressing MPO and H3 citr in saline (I) , LPS (J) and LPS + atenolol (K) groups. Representative pictures of Iba1 + microglia (green) labeling with citrullinated histone H3 (H3 citr) (red). The nucleus of cells was labeled with DAPI (in blue) for saline (L) and LPS (M) groups. Saline n=16, atenolol n=6, LPS n=13, LPS + atenolol n=10. ** p



Sivelestat decreased the production of ETs induced by LPS. Quantification of mean fluorescence intensities (MFIs) of extracellular trap markers MPO (A) , H3 citr (B) , Sytox (C) and TFAM (D) for saline (blue), sivelestat (turquoise), LPS (red) and LPS + sivelestat (khaki) groups in live neutrophils. Quantification of MFI of extracellular traps markers MPO (E) , H3 citr (F) , Sytox (G) and TFAM (H) for saline (blue), sivelestat (turquoise), LPS (red) and LPS + sivelestat (khaki) groups in live microglia. Representative dot plot of live neutrophils co-expressing MPO and H3 citr in saline (I) , LPS (J) and LPS + sivelestat (K) . Representative dot plot of living microglia co-expressing MPO and H3 citr in saline (L) , LPS (M) and LPS + sivelestat (N) groups. Saline n=16, sivelestat n=6, LPS n=13, LPS + sivelestat n=8. * p

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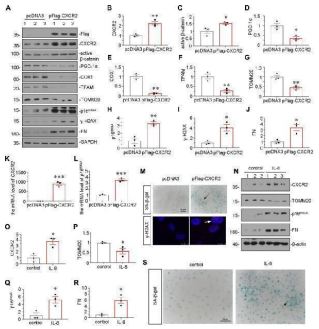
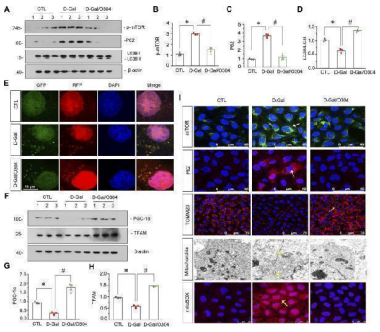


CXCR2 promotes mitochondrial dysfunction and tubular senescence in vitro . (A–J) Representative micrographs of western blot and quantitative statistical data show renal expression of (B) CXCR2 (C) active beta-catenin, (D) PGC-1alpha, (E) COX1, (F) TFAM, (G) TOMM20, (H) p16 INK4A , (I) gamma-H2AX and (J) FN in each group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, versus the pcDNA3 group ($n = 3$). (K,L) Graphical representations show the relative mRNA level of (K) CXCR2 and (L) p16 INK4A in different groups. *** $p < 0.001$ versus the pcDNA3 group ($n = 3$). (M) Representative micrographs show SA-beta-gal staining and gamma-H2AX expression in different groups. Frozen sections were performed by SA-beta-gal and gamma-H2AX immunofluorescence staining. Arrows indicates positive staining. Scale bar = 50 or 10 um. (N–R) Representative micrographs of western blot and quantitative statistical data show protein levels of (O) CXCR2, (P) TOMM20, (Q) p16 INK4A and (R) FN in each group. * $p < 0.05$ versus the control group ($n = 3$). (S) Representative micrographs show SA-beta-gal staining in each group. Frozen renal sections were performed by SA-beta-gal staining. Arrows indicate positive staining. Scale bar, 50 um.Index in PubMed under a CC BY license. PMID: 35592244



O304 could promote autophagy and alleviate mitochondrial dysfunction in vitro . (A–D) Representative western blot showing the expression of p-mTOR, P62, and LC3B in different groups. HKC-8 cells were stimulated by D-Gal (10 mg/ml) for 60 h with or without O304 (50 nM). * $p < 0.05$ versus control (Ctrl) cells; # $p < 0.05$ versus D-Gal-treated group alone ($n = 3$). (E) Representative micrographs show that O304 promoted acidic-pH LC3B-positive autophagolysosomes (red fluorescence). HKC-8 cells were pre-transfected with lentiviruses expressing RFP-GFP-LC3B for 12 h, and were then stimulated by D-Gal (10 mg/ml) for 60 h with or without O304 (50 nM). Natural-pH LC3B-positive autophagosomes (green fluorescence) and acidic-pH LC3B-positive autophagolysosomes (red fluorescence) were detected. (F–H) Representative western blot and quantitative data showing the expression of PGC-1alpha and TFAM. * $p < 0.05$ versus control group; # $p < 0.05$ versus D-Gal-treated group alone ($n = 3$). (I) Representative micrographs showing the expression of mTOR, P62, TOMM20 and mitochondrial ROS production in different groups. HKC-8 cells were seeded on coverslips and stimulated by D-Gal (10 mg/ml) for 60 h with or without O304 (50 nM). The cells were immunostained with mitoSOX probe or antibodies against mTOR, P62 and TOMM20, respectively. Representative transmission electron microscopy (TEM) micrographs (middle) showing O304 protects the normal structure of mitochondria in HKC-8 cells. For mTOR, P62, TOMM20 and mitoSOX staining, arrows indicate positive staining. For TEM analyses, arrowheads indicate abnormal-shaped mitochondria.Index in PubMed under a CC BY license. PMID: 35308246

Western blot analysis of mtTFA using anti-mtTFA antibody (PB9447). Electrophoresis was performed on a 12% SDS-

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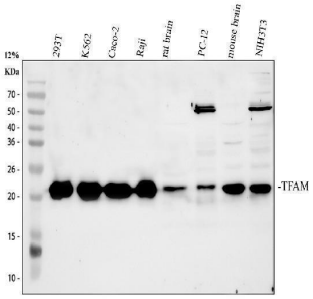
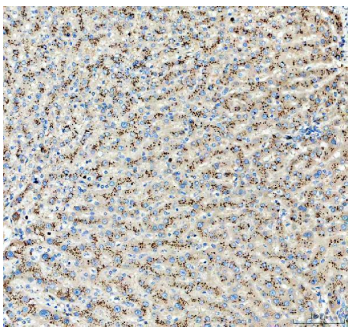
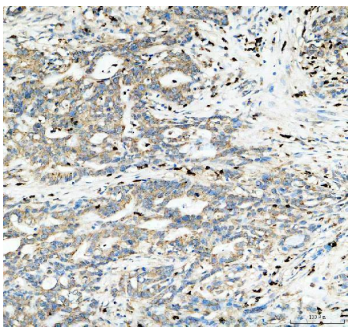


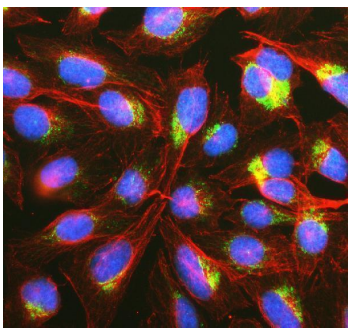
PAGE gel at 80V (Stacking gel) / 120V (Resolving gel) for 2 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human 293T whole cell lysates, Lane 2: human K562 whole cell lysates, Lane 3: human Caco-2 whole cell lysates, Lane 4: human Raji whole cell lysates, Lane 5: rat brain tissue lysates, Lane 6: rat PC-12 whole cell lysates, Lane 7: mouse brian tissue lysates, Lane 8: mouse NIH/3T3 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 rabbit anti-mtTFA antigen affinity purified polyclonal antibody (PB9447) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody (Catalog # BA1054) at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an ECL Plus Western Blotting Substrate (Catalog # AR1196-200) with Tanon 5200 system. A specific band was detected for mtTFA at approximately 24 kDa. The expected band size for mtTFA is at 29 kDa.



IHC analysis of mtTFA using anti-mtTFA antibody (PB9447). mtTFA was detected in a paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-mtTFA Antibody (PB9447) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.



IHC analysis of mtTFA using anti-mtTFA antibody (PB9447). mtTFA was detected in a paraffin-embedded section of human pancrease cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-mtTFA Antibody (PB9447) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.



IF analysis of mtTFA using anti-mtTFA antibody (PB9447) and anti-Tubulin Alpha antibody (M03989-3). mtTFA was detected in immunocytochemical section of U2OS cell. 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 ug/mL rabbit anti-mtTFA Antibody (PB9447) and mouse anti-Tubulin Alpha antibody (M03989-3) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) and

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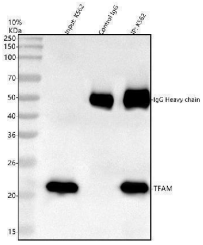
Cy3 Conjugated Goat Anti-Mouse IgG (BA1031) were used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



Immunoprecipitating (IP) mtTFA in K562 whole cell lysate. Western blot analysis of mtTFA using anti-mtTFA antibody (PB9447); Lane 1: K562 whole cell lysates (30ug); Lane 2: Rabbit control IgG instead of anti-mtTFA antibody in K562 whole cell lysate; Lane 3: anti-mtTFA antibody (2ug) + K562 whole cell lysate (500ug). After electrophoresis, proteins were transferred to a membrane. Then the membrane was incubated with rabbit anti-mtTFA antigen affinity purified polyclonal antibody (PB9447) at a dilution of 0.5 ug/mL and probed with a goat anti-rabbit IgG-HRP secondary antibody (Catalog # BA1054). The signal is developed using ECL Plus Western Blotting Substrate (Catalog # AR1196-200). A specific band was detected for mtTFA at approximately 24 kDa. The expected band size for mtTFA is at 29 kDa.

3 Publications Citing This Product

1. PubMed ID: -, Shen W,Jia N,Miao J,Chen S,Zhou S,Meng P,Zhou X,Tang L,Zhou L:Penicilliumin B Protects against Cisplatin-Induced Renal Tubular Cell Apoptosis through Activation of AMPK-Induced Autophagy and Mitochondrial Biogenesis.Kidney Dis 2021.doi:10.1159/000514657

2. PubMed ID: 24137381, KAIMING WU, ZHENXIAN ZHAO, YINGLIAN XIAO, JIANJUN PENG, JIANHUI CHEN and YULONG HE MOLECULAR MEDICINE REPORTS 14: 5475-5480, 2016 DOI: 10.3892/mmr.2016.5955 Roles of mitochondrial transcription factor A and microRNA□590□3p in the development of...

3. PubMed ID: 27878255, Roles of mitochondrial transcription factor A and microRNA-590-3p in the development of colon cancer

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