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Anti-mtTFA TFAM Antibody Picoband™ (monoclonal, 4D9)

Catalog # ABO14894

Product Information

Application	WB, IHC, FC
Primary Accession	Q00059
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-mtTFA TFAM Antibody Picoband™ (monoclonal, 4D9) . Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500 □g/ml.

Additional Information

Gene ID	7019
Other Names	Transcription factor A, mitochondrial, mtTFA, Mitochondrial transcription factor 1, MtTF1, Transcription factor 6, TCF-6, Transcription factor 6-like 2, TFAM (HGNC:11741), TCF6, TCF6L2
Calculated MW	29097
Application Details	Western blot, 1-2 □g/ml, Human Immunohistochemistry (Paraffin-embedded Section), 0.5-1 □g/ml, Human, By Heat Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Subcellular Localization	Mitochondrion; mitochondrion nucleoid
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na_2HPO_4, 0.05mg NaN_3.
Clone Names	Clone: 4D9
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.
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

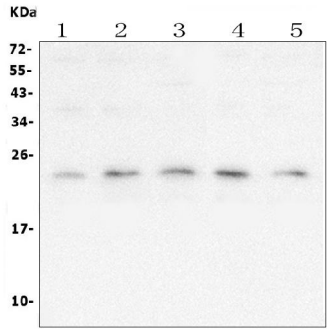
Name	TFAM (HGNC:11741)
Synonyms	TCF6, TCF6L2
Function	Binds to the mitochondrial light strand promoter and functions in mitochondrial transcription regulation (PubMed:29445193, PubMed:32183942). Component of the mitochondrial transcription initiation complex, composed at least of TFB2M, TFAM and POLRMT that is required for basal transcription of mitochondrial DNA (PubMed:29149603). In this complex, TFAM recruits POLRMT to a specific promoter whereas TFB2M induces structural changes in POLRMT to enable promoter opening and trapping of the DNA non-template strand (PubMed:20410300). Required for accurate and efficient promoter recognition by the mitochondrial RNA polymerase (PubMed:22037172). Promotes transcription initiation from the HSP1 and the light strand promoter by binding immediately upstream of transcriptional start sites (PubMed:22037172). Is able to unwind DNA (PubMed:22037172). Bends the mitochondrial light strand promoter DNA into a U-turn shape via its HMG boxes (PubMed:1737790). Required for maintenance of normal levels of mitochondrial DNA (PubMed:19304746, PubMed:22841477). May play a role in organizing and compacting mitochondrial DNA (PubMed:22037171).
Cellular Location	Mitochondrion. Mitochondrion matrix, mitochondrion nucleoid

Background

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.

Images

Figure 1. Western blot analysis of TFAM using anti ZO-1 antibody (M01119-1).
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 HEK293 tissue 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: human A549 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-TFAM antigen affinity purified polyclonal antibody (Catalog # M01119-1) 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 TFAM at approximately 40KD. The expected band size for TFAM is at 40KD.

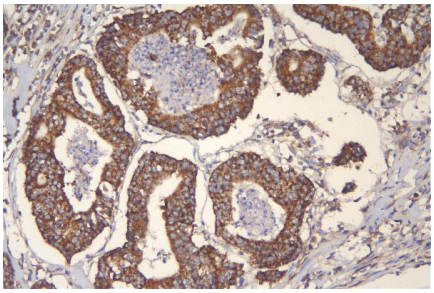


Figure 2. IHC analysis of TFAM using anti-TFAM antibody (M01119-1).
TFAM was detected in paraffin-embedded section of human rectum cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-TFAM Antibody (M01119-1) 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.

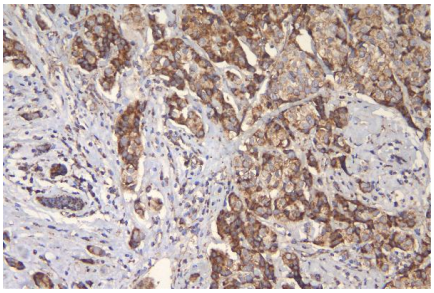


Figure 3. IHC analysis of TFAM using anti-TFAM antibody (M01119-1).
TFAM was detected in paraffin-embedded section of human Breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-TFAM Antibody (M01119-1) 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.

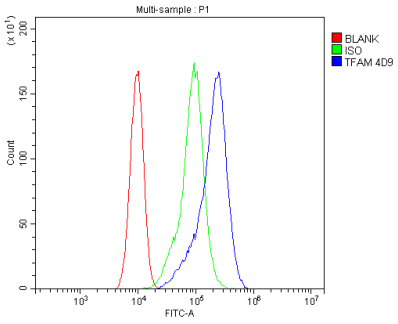


Figure 4. Flow Cytometry analysis of CACO-2 cells using anti-FAM antibody (M01119-1).
Overlay histogram showing CACO-2 cells stained with M01119-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-FAM Antibody (M01119-1, 1 µg/1x10^6 cells) for 30 min at 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.