

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

Catalog # ABO14894

#### Specification

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

Application Primary Accession Host Isotype Reactivity Clonality Format Description WB, IHC, FC <u>000059</u> Mouse Mouse IgG2b Human Monoclonal Lyophilized

Anti-mtTFA TFAM Antibody Picoband<sup>™</sup> (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  $\mu$ g/ml.

## Anti-mtTFA TFAM Antibody Picoband<sup>™</sup> (monoclonal, 4D9) - 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 (<a href="http://www.genenames.org/cgi-bin/gene\_symbol\_report?hgnc\_id=11741" target="\_blank">HGNC:11741</a>), TCF6, TCF6L2

Calculated MW 40 kDa KDa

Application Details Western blot, 1-2  $\mu$ g/ml, Human<br> Immunohistochemistry (Paraffin-embedded Section), 0.5-1  $\mu$ g/ml, Human, By Heat<br/>br> Flow Cytometry, 1-3  $\mu$ g/1x10^6 cells, Human<br>

**Subcellular Localization** Mitochondrion; mitochondrion nucleoid

**Contents** Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na<sub>2</sub>HPO<sub>4</sub>, 0.05mg NaN<sub>3</sub>.

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.

## Anti-mtTFA TFAM Antibody Picoband<sup>™</sup> (monoclonal, 4D9) - Protein Information

Name TFAM (HGNC:11741)

Synonyms TCF6, TCF6L2

Function

Binds to the mitochondrial light strand promoter and functions in mitochondrial transcription regulation (PubMed: <a href="http://www.uniprot.org/citations/29445193" target="\_blank">29445193</a>, PubMed:<a href="http://www.uniprot.org/citations/32183942" target=" blank">32183942</a>). 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:<a href="http://www.uniprot.org/citations/29149603" target=" blank">29149603</a>). 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:<a href="http://www.uniprot.org/citations/20410300" target=" blank">20410300</a>). Required for accurate and efficient promoter recognition by the mitochondrial RNA polymerase (PubMed: <a href="http://www.uniprot.org/citations/22037172" target=" blank">22037172</a>). Promotes transcription initiation from the HSP1 and the light strand promoter by binding immediately upstream of transcriptional start sites (PubMed:<a href="http://www.uniprot.org/citations/22037172" target="\_blank">22037172</a>). Is able to unwind DNA (PubMed:<a href="http://www.uniprot.org/citations/22037172" target=" blank">22037172</a>). Bends the mitochondrial light strand promoter DNA into a U-turn shape via its HMG boxes (PubMed: <a href="http://www.uniprot.org/citations/1737790" target=" blank">1737790</a>). Required for maintenance of normal levels of mitochondrial DNA (PubMed:<a href="http://www.uniprot.org/citations/19304746" target=" blank">19304746</a>, PubMed:<a href="http://www.uniprot.org/citations/22841477" target=" blank">22841477</a>). May play a role in organizing and compacting mitochondrial DNA (PubMed:<a href="http://www.uniprot.org/citations/22037171" target=" blank">22037171</a>).

#### **Cellular Location**

Mitochondrion. Mitochondrion matrix, mitochondrion nucleoid

## Anti-mtTFA TFAM Antibody Picoband<sup>™</sup> (monoclonal, 4D9) - 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-mtTFA TFAM Antibody Picoband<sup>™</sup> (monoclonal, 4D9) - 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  $\mu$ 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 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  $\mu$ g/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  $\mu$ g/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  $\mu$ 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-mtTFA TFAM Antibody Picoband™ (monoclonal, 4D9) - 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.