



ab123455

mtTFA (TFAM) Human

ELISA Kit

Instructions for Use

For the quantitative measurement of Human mtTFA protein.

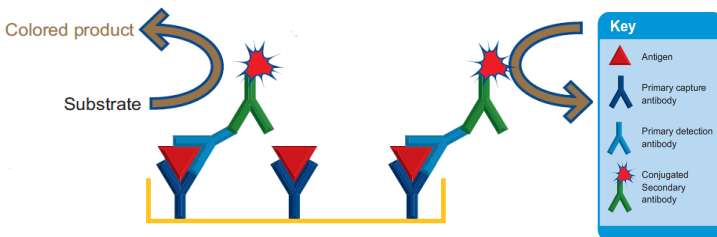
This product is for research use only and is not intended for diagnostic use.

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1. Introduction

Principle: ab123455 mtTFA Human ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human mtTFA (TFAM) in cell and tissue lysates. The assay employs an antibody specific for human mtTFA coated onto well plate strips. Standards and samples are pipetted into the wells and analyte present in the sample is bound to the wells by the immobilized antibody. The wells are washed and an anti- mtTFA primary detector antibody is added. After washing away unbound primary detector antibody, HRP-conjugated secondary detector antibody specific for the primary detector antibody is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of analyte bound. The developing blue color is measured at 600 nm. Optionally the reaction can be stopped by adding hydrochloric acid which changes the color from blue to yellow and the intensity can be measured at 450 nm.



Background: mtTFA (mitochondrial transcription factor A or TFAM) is a nuclear DNA encoded mitochondrial protein involved with mitochondrial DNA transcription and packaging. mtTFA binds to the mitochondrial light strand promoter and functions in mitochondrial transcription regulation. mtTFA is required for accurate and efficient promoter recognition by the mitochondrial RNA polymerase. mtTFA is required for the maintenance of normal levels of mitochondrial DNA and may play a role in organizing and compacting mitochondrial DNA. mtTFA is able to unwind and bend DNA.

2. Assay Summary

Equilibrate all reagents to room temperature. Prepare all the reagents, samples, and standards as instructed.



Add 50 μL standard or sample to each well used. Incubate 2 hours at room temperature.



Aspirate and wash each well two times. Add 50 μL prepared 1X Detector Antibody to each well. Incubate 1 hour at room temperature.



Aspirate and wash each well two times. Add 50 μL prepared 1X HRP Label to each well. Incubate 1 hour at room temperature.



Aspirate and wash each well three times. Add 100 μL TMB Development Solution to each well. Immediately begin recording the color development for 10 minutes at 600 nm. Alternatively add a Stop solution at a user-defined time and read at 450 nm.

3. Kit Contents

Item	Quantity
10X Wash Buffer	40 mL
Extraction Buffer	15 mL
2X Blocking Buffer	30 mL
TMB Development Solution	12 mL
10X mtTFA Detector Antibody	1 mL
10X HRP Label	1 mL
mtTFA Microplate (12 x 8 antibody coated well strips)	96 wells
Human mtTFA standard (100 ng)	1 vial

4. Storage and Handling

Store all components at 4 °C. This kit is stable for at least 6 months from receipt. After reconstitution the standard should be stored at -80 °C. Unused microplate strips should be returned to the pouch containing the desiccant and resealed.

5. Additional Materials Required

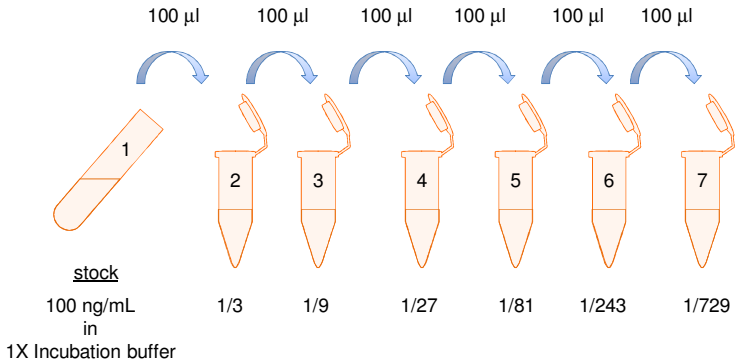
- Microplate reader capable of measuring absorbance at 600 nm (or 450 nm after addition of Stop solution - not supplied).
- Method for determining protein concentration (BCA assay recommended).
- Deionized water
- Multi and single channel pipettes
- PBS (1.4 mM KH_2PO_4 , 8 mM Na_2HPO_4 , 140 mM NaCl, 2.7 mM KCl, pH 7.3)
- Tubes for standard dilution
- Stop solution (optional) – 1N Hydrochloric acid
- Optional plate shaker for all incubation steps
- Well plate cover or seals

6. Preparation of Reagents

- 6.1 Equilibrate all reagents to room temperature (18-25°C) before use.
- 6.2 Prepare 1X Wash Buffer by adding 32 mL 10X Wash Buffer to 288 mL nanopure water. Mix by gentle inversion.

- 6.3 Prepare 1X Incubation Buffer by adding 30 mL 2X Blocking Buffer and 6 mL 10X Wash Buffer to 24mL nanopure water. Mix by gentle inversion. Unused 1X Incubation Buffer may be stored at -20°C for 6 months after performing the ELISA.
- 6.4 Prepare the 1X Detector Antibody by diluting the 10X mtTFA Detector Antibody 10-fold with 1X Incubation Buffer immediately prior to use. Prepare 0.5 mL for each 8 well strip used.
- 6.5 Prepare the 1X HRP Label by diluting the stock 10X HRP Label 10-fold with 1X Incubation Buffer immediately prior to use. Prepare 0.5 mL for each 8 well strip used.
- 6.6 Reconstitute the Human mtTFA standard (100 ng) with 1 mL 1X Incubation buffer by pipetting. Allow to sit for 10 minutes and repeat pipetting to ensure thorough reconstitution. This 100 ng/mL stock of standard material is then used to generate a standard curve in labeled tubes as described below (6.7). Any remaining stock material can be stored at -80°C for 6 months.
- 6.7 To prepare serially diluted standards, label six tubes #2-7. Label the reconstituted stock human mtTFA standard from step 6.6 tube #1. Add 200 μ L of 1X Incubation buffer to each of tubes #2 through #7. Transfer 100 μ L from tube #1 to tube #2. Mix thoroughly. With a fresh pipette tip transfer 100 μ L

from #2 to #3. Mix thoroughly. Repeat for Tubes #4 through #7. Use 1X Incubation buffer as the zero standard tube labeled #8. Use fresh standards for each assay.



7. Sample Preparation

Note: Extraction buffer can be supplemented with phosphatase inhibitors, PMSF and protease inhibitor cocktail prior to use. Supplements should be used according to manufacturer's instructions.

7.1 Preparation of extract from cell pellets

7.1.1 Collect non adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. If the adherent cells are detaching, as typical for apoptotic cells, collect both floating cells and

remaining adherent cells. Typical centrifugation conditions for cells are 500 x g for 10 min at 4°C.

- 7.1.2 Rinse cells twice with PBS.
- 7.1.3 Solubilize cell pellet at 2×10^7 /mL in Extraction Buffer.
- 7.1.4 Incubate on ice for 20 minutes. Centrifuge at 16,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C for 6 months. The sample protein concentration in the extract may be quantified using a protein assay.

7.2 Preparation of extracts from tissue homogenates

- 7.2.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).
- 7.2.2 Suspend the homogenate to 10 mg/mL in PBS.
- 7.2.3 Solubilize the homogenate by adding equal volume of Extraction Buffer to the homogenate.
- 7.2.4 Incubate on ice for 20 minutes. Centrifuge at 16,000 x g, for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

The sample should be diluted to within the working range of the assay in 1X Incubation Buffer. As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Data Analysis.

8. Assay Procedure

Equilibrate all reagents and samples to room temperature before use. It is recommended all samples and controls be assayed in duplicate.

- 8.1 Prepare all reagents, working dilutions of controls and samples as directed in the previous sections.
- 8.2 Remove unused microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and seal.
- 8.3 Add 50 μ L of each serially diluted human mtTFA standards (tubes 2 – 8) per well. It is recommended to include a dilution series of a control (normal) sample as a reference. Also include a 1X Incubation buffer as a zero standard. Cover/seal the plate and incubate for 2 hours at room temperature. If available use a plate shaker for all incubation steps at 300 rpm.

- 8.4 Aspirate each well and wash, repeat this once more for a total of **two** washes. Wash by aspirating or decanting from wells then dispensing 300 μ L 1X Wash Buffer into each well as described above. Complete removal of liquid at each step is essential to good performance. After the last wash, remove the remaining buffer by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid.
- 8.5 Immediately before use prepare sufficient (0.5 mL/strip used) 1X Detector Antibody (step 6.4) in 1X Incubation Buffer. Add 50 μ L 1X Detector antibody to each well used. Cover/seal the plate and incubate for 1 hour at room temperature. If available use a plate shaker for all incubation steps at 300 rpm.
- 8.6 Repeat the aspirate/wash procedure above.
- 8.7 Immediately before use prepare sufficient (0.5 mL/strip used) 1X HRP Label in 1X Incubation Buffer (step 6.5). Add 50 μ L 1X HRP Label to each well used. Cover/seal the plate and incubate for 1 hour at room temperature. If available use a plate shaker for all incubation steps at 300 rpm.
- 8.8 Repeat the aspirate/wash procedure above, however, performing a total of **three** washes.

- 8.9 Add 100 μL TMB Development Solution to each empty well and immediately record the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode:	Kinetic
Wavelength:	600 nM
Time:	up to 15 min.
Interval:	20 sec. - 1 min.
Shaking:	Shake between readings

Alternative– In place of a kinetic reading, at a **user defined**, time record the endpoint OD data at (i) 600 nm or (ii) stop the reaction by adding 50 μL stop solution (1N HCl) to each well and record the OD at 450 nm.

- 8.10 Analyze the data as described below.

9. Data Analysis

Average the duplicate positive controls readings and plot against their concentrations after subtracting the zero standard reading. Draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be

examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Read relative mtTFA concentrations for unknown samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted in and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

TYPICAL STANDARD CURVE - *For demonstration only.*

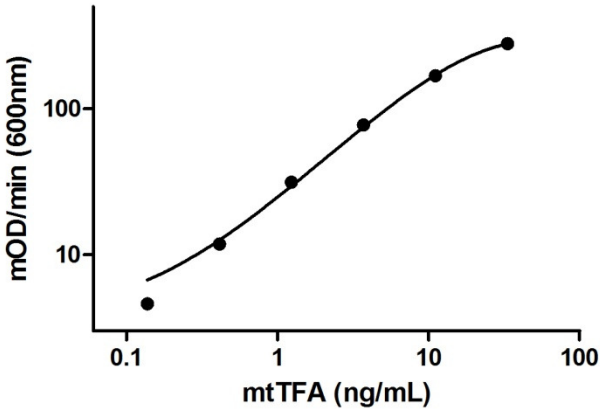


Figure 1. Example standard curve. A dilution series of recombinant mtTFA (ab103784) in the working range of the assay.

TYPICAL EXPERIMENTAL RESULTS - *For demonstration only.*

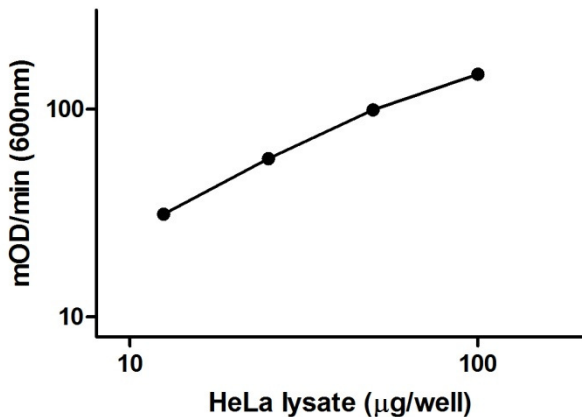


Figure 2. Example HeLa dilution series and working range.

Typical working ranges for cell extracts	
Sample Type	Range
HeLa, HEK 293T	0.01 -0.2 mg/mL

SENSITIVITY

Determined minimum detectable dose = 0.05 ng/mL mtTFA standard

LINEARITY OF DILUTION

Linearity of dilution determined by comparing dilution series of extracts prepared from HeLa cells (starting extract concentration is 100 µg/mL).

Sample	% Expected Value
undiluted	100
1:2	115
1:4	121
1:8	107

REPRODUCIBILITY

Parameter	CV%
Intra (n=6)	4.0
Inter (n=3)	10.2

10. Specificity

Species– Human reactive. Non-reactive with mouse and rat. Others untested.

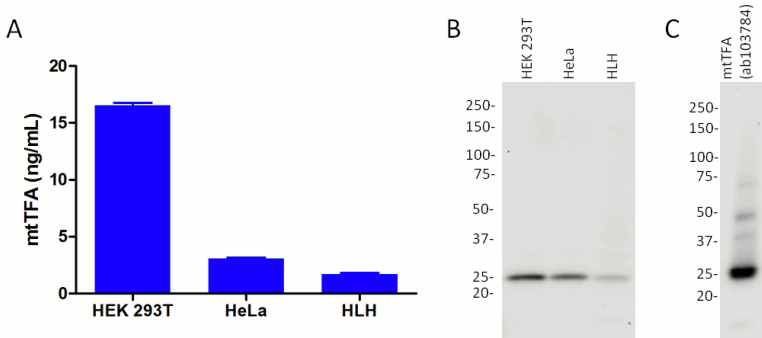


Figure 3. Demonstration of assay specificity. (A) 25 μ g/mL HEK 293T and HeLa cell lysates and Human Liver Homogenate (HLH) were assayed by the mtTFA ELISA (ab123455) and mtTFA concentration was interpolated from the standard curve. (B & C) Western blots using the mtTFA ELISA detector antibody (ab119684) at 1 μ g/mL: (B) 20 μ g of each lysate and (C) 30ng of mtTFA standard (ab103784).

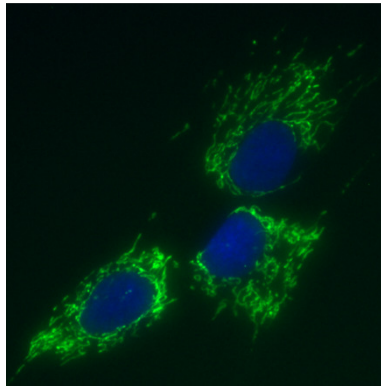


Figure 4. Immunocytochemistry image of mtTFA ELISA detector antibody (ab119684) at 1 µg/mL in human HDFn cells.

11. Troubleshooting

Problem	Cause	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Low mtTFA concentration in sample	Use appropriate positive control/treatment to induce apoptosis/mtTFA
	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep substrate solution protected from light

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