

INTENDED USE

This human thrombin activatable fibrinolysis inhibitor (TAFI) antigen assay is intended for the quantitative determination of total TAFI antigen in human plasma and serum. **For research use only.**

BACKGROUND

TAFI, also known as carboxypeptidase B2 (PCB2), plasma carboxypeptidase-B (pCB), and carboxypeptidase-U (CPU), is a 401 amino acid 58 kDa plasma glycoprotein that is activated by thrombin. Activated TAFI (TAFIa) cleaves fibrin carboxy-terminal lysines reducing plasminogen and tPA binding sites resulting in downregulation of fibrinolysis [1]. Although mice made genetically deficient for TAFI do not show a marked phenotype, change in human plasma TAFI level has been associated with many cardiovascular disorders [2-4].

ASSAY PRINCIPLE

Human TAFI will bind to the affinity purified capture antibody coated on the microtiter plate. TAFI and TAFIa will react with the antibody on the plate. After appropriate washing steps, biotinylated primary antibody binds to the captured protein. Excess primary antibody is washed away, and bound antibody is reacted with horseradish peroxidase conjugated streptavidin. TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of human TAFI. Color development is directly proportional to the concentration of total TAFI in the samples.

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-human TAFI antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Human TAFI standard:** 1 vial lyophilized standard
- **Anti-human TAFI primary antibody:** 1 vial lyophilized polyclonal antibody
- **Horseradish peroxidase-conjugated streptavidin:** 1 vial concentrated HRP labeled streptavidin
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standard and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H₂SO₄ or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- **TBS buffer:** 0.1M Tris, 0.15M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water

SAMPLE COLLECTION

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard

Reconstitute standard according to the C of A. Agitate gently to completely dissolve contents.

Dilution table for preparation of human TAFI standard:

TAFI concentration (ng/ml)	Dilutions
500	from vial
250	500 μl (BB) + 500 μl (500ng/ml)
100	600 μl (BB) + 400 μl (250ng/ml)
50	500 μl (BB) + 500 μl (100ng/ml)
20	600 μl (BB) + 400 μl (50ng/ml)
10	500 μl (BB) + 500 μl (20ng/ml)
5	500 μl (BB) + 500 μl (10ng/ml)
2	600 μl (BB) + 400 μl (5ng/ml)
1	500 μl (BB) + 500 μl (2ng/ml)
0	500 μl (BB) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition

Remove microtiter plate from bag and add 100 μl TAFI standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 μl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures total human TAFI antigen in the 1-500 ng/ml range. If the unknown is thought to have TAFI levels greater than 500ng/ml, dilutions may be made in blocking buffer before use. A 1:100-1:1,000 dilution for normal human plasma or serum is suggested for best results.

Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100 μl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 μl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Streptavidin-HRP Addition

Briefly centrifuge vial before opening. **See C of A for lot specific dilution instructions.** Add 100 μl of your dilution to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 μl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 100 μl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50 μl of 1N H_2SO_4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

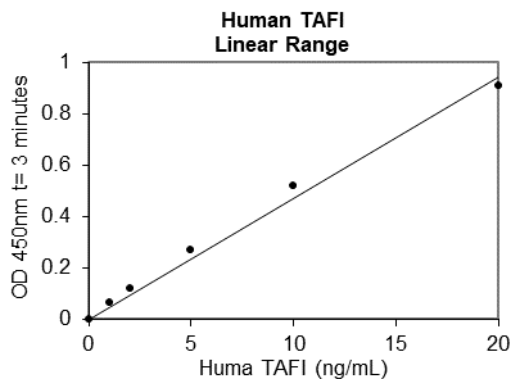
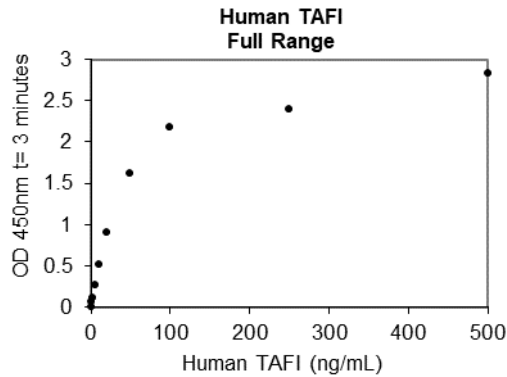
Measurement

Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

Plot A_{450} against the amount of human TAFI in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four-parameter logistic (4PL) curve fit. The amount of TAFI in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):



EXPECTED VALUES

The average concentration of TAFI in normal human plasma is 4.2 $\mu\text{g/ml}$ [5].

PERFORMANCE CHARACTERISTICS

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration. **See C of A for lot specifications.**

Intra-assay Precision: These studies are currently in progress. Please contact us for more information.

Inter-assay Precision: These studies are currently in progress. Please contact us for more information.

Recovery: These studies are currently in progress. Please contact us for more information.

Linearity: These studies are currently in progress. Please contact us for more information.

Sample Values: Samples were evaluated for the presence of the antigen at varying dilutions.

Sample Type	Dilution	Mean ($\mu\text{g/mL}$)
Citrate Plasma	1:500	4.0
	1:1,000	4.4
Serum	1:500	8.9

Specificity: These studies are currently in progress. Please contact us for more information.

REFERENCES

1. Zhao L, *et al.*: J Biol Chem. 2003, 278:32359-32366.
2. Bouma BN & Mosnier LO: Pathophysiol Haemost Thromb. 2003/2004, 33:375-381.
3. Bajzar L, *et al.*: J Biol Chem. 1996, 271:16603-16608.
4. Van Tilburg NH, *et al.*: Blood 2000, 95:2855-2859.
5. Bajzar L, *et al.*: Blood 1996, 88:2093-2100.

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and should be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

Example of ELISA Plate Layout

96 Well Plate: 20 Standard wells, 76 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	250 ng/ml	500 ng/ml		
B	0	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	250 ng/ml	500 ng/ml		
C												
D												
E												
F												
G												
H												