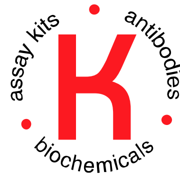


KAMIYA BIOMEDICAL COMPANY

Human TAFI ELISA

For the quantitative determination of TAFI in human plasma

Cat. No. KT-453

For research use only, not for use in diagnostic procedures.

Revised 111108

PRODUCT INFORMATION

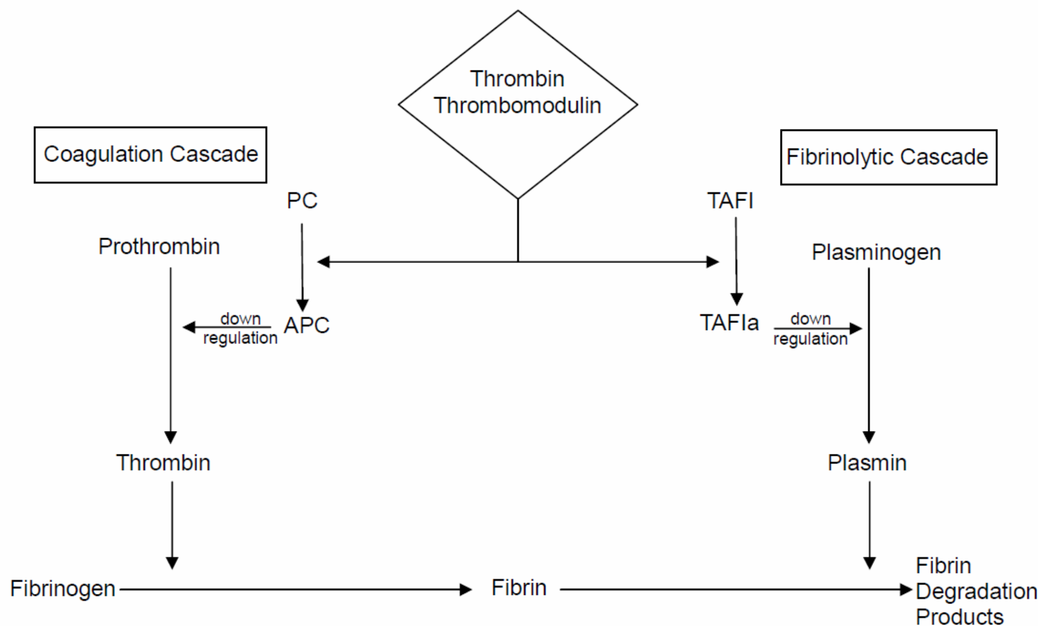
Human TAFI ELISA
Cat. No. KT-453

PRODUCT
The K-ASSAY® Human TAFI ELISA is for the quantitative determination of TAFI in human plasma.

BACKGROUND
When the coagulation and fibrinolytic cascades are properly regulated there is a balance between fibrin deposition and removal. It is this balance that prevents catastrophic blood loss upon injury to the vascular system. When the coagulation and fibrinolytic cascades are not in balance, excessive blood loss or blood clotting can result. The thrombin-thrombomodulin complex can activate protein C to down regulate the coagulation cascade and increase the time needed for blood clot formation (figure 1). The thrombin-thrombomodulin complex can also activate thrombin-activatable fibrinolysis inhibitor (TAFI) to down regulate the fibrinolytic cascade and decrease the time needed for blood clot formation. The activation of one cascade suppresses the activities of the other cascade. When the coagulation and fibrinolytic cascades are not properly balanced, pathophysiologic consequences occur in the form of bleeding and thrombosis. The most common thrombotic events are heart attacks and strokes. TAFI, also known as procarboxypeptidase R (proCPR), procarboxypeptidase U (proCPU), and pro-plasma carboxypeptidase B (pro-pCPB), is a 60 kDa protein synthesized in the liver and found circulating in the blood as a zymogen. Activated TAFI (TAFIa) suppresses fibrinolysis by removal of the carboxy-terminal lysine and arginine residues from partly degraded fibrin polymers, preventing the binding of the fibrinolytic components plasminogen and tissue-type plasminogen activator to fibrin.

This Human TAFI ELISA is designed to measure the concentration of TAFI in human plasma using 2 monoclonal antibodies, 2A16 and 10G1 targeted to different epitopes of TAFI.

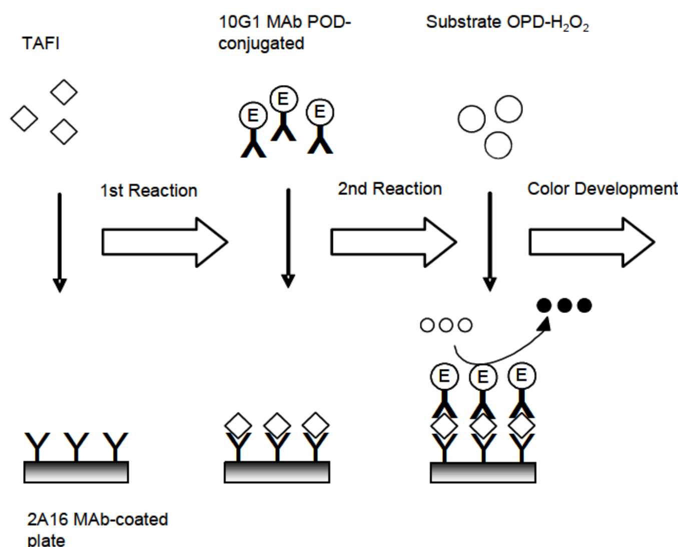
Figure 1. Balance Between Coagulation and Fibrinolytic Cascade[1]



PRINCIPLE

The principle of the assay is shown in Figure 2. Calibrators and samples are incubated in a 96-well microtiter plate coated with monoclonal antibody 2A16. The plate is incubated for 1 hour at room temperature followed by 3 washes. Secondary monoclonal antibody, 10G1 labeled with peroxidase (POD), is added to each well and incubated for 1 hour at room temperature. After incubation, the plate is washed and the substrate added. The enzymatic reaction is stopped by the addition of stop solution and absorbance is measured at 492 nm. The amount of color development is directly proportional to the concentration of human TAFI in the test sample.

Figure 2. Assay Principle



COMPONENTS

- Microtiter Plate: 96-well plate coated with 2A16 mAb, provided in a resealable foil pouch
- 5X Sample Dilution Buffer: phosphate buffered saline with protein stabilizer and 10 mM EDTA, 40 mL
- 6X Wash Buffer: phosphate buffered saline with 0.3% Tween 20, 50 mL
- Calibrator (lyophilized): Human plasma containing 48 ng TAFI
- Detection Antibody: 10G1 mAb-horseradish peroxidase (POD)-conjugated, 30 µL
- 30% H_2O_2 (Hydrogen Peroxide): 30 µL
- OPD Substrate Tablet: 1 Tablet
- Color Developing Buffer: 33 mM citrate and 67 mM sodium phosphate (pH 5.0), 20 mL
- Stop Solution: 2N Sulfuric acid, 20 mL

Materials or Equipment required but not provided

- Graduated cylinder
- Micropipette(s) and disposable pipette tips
- 96-well plate or manual strip washer
- Paper towels or absorbent paper
- Plate reader capable of measuring absorbance at a wavelength of 492 nm. Reference filter at 630 nm is optional.
- Sterile containers such as microtubes, 10 and 25 mL tubes
- Refrigerator 4ºC
- Ice bath or equivalent
- 37ºC water bath or equivalent
- De-ionized water or equivalent

PREPARATION OF REAGENTS

Reagents should be diluted just prior to use.

Do not mix reagents from different kits unless they have the same lot number.

A. **1X Sample Dilution Buffer**
Prepare 1X Wash Solution by mixing 40 mL of the 5X Sample Dilution Buffer with 160 mL of de-ionized water or equivalent. Mix well. After preparation, store 1X Sample Dilution Buffer at 4°C. The 1X buffer is stable for 1 month at 4°C.

B. **6X Wash Buffer**
Crystals appearing in the 6X Wash Buffer will be solubilized during preparation of the 1X Wash Buffer. Prepare 1X Wash Buffer by mixing 50 mL of the 6X Wash Buffer with 250 mL of de-ionized water or equivalent. Mix well. After preparation, the 1X Wash Buffer is stable for 1 month at 4°C.

C. **Plasma Calibrators**
Add 1 mL of 1X Sample Dilution Buffer to the lyophilized calibrator vial to prepare 48 ng/mL TAFI. The reconstituted calibrator is 2-fold serial diluted in 1X Sample Dilution Buffer to prepare 24, 12, 6, 3, 1.5, and 0.75 ng/mL calibrators. Undiluted calibrator and 1X Sample Dilution Buffer will be used for 48 ng/mL and 0 ng/mL, respectively. The diluted calibrators will be used to generate a calibration curve. Dilutions should be performed in an ice bath and kept on ice until used. The diluted calibrators should be used within 30 minutes of preparation.

D. **Detection Antibody**
Transfer the entire volume (30 µL) of the Detection Antibody to a sterile 10 mL tube. Dilute the Detection Antibody with 6 mL of 1X Sample Dilution Buffer.

E. **OPD-H_2O_2 Color Development Reagent**
Dissolve the OPD tablet in 20 mL of the Color Development Buffer. Once dissolved, add 10 µL of 30% H_2O_2. The color development reagent should be prepared just prior to use. Wear gloves when preparing and handling this reagent.

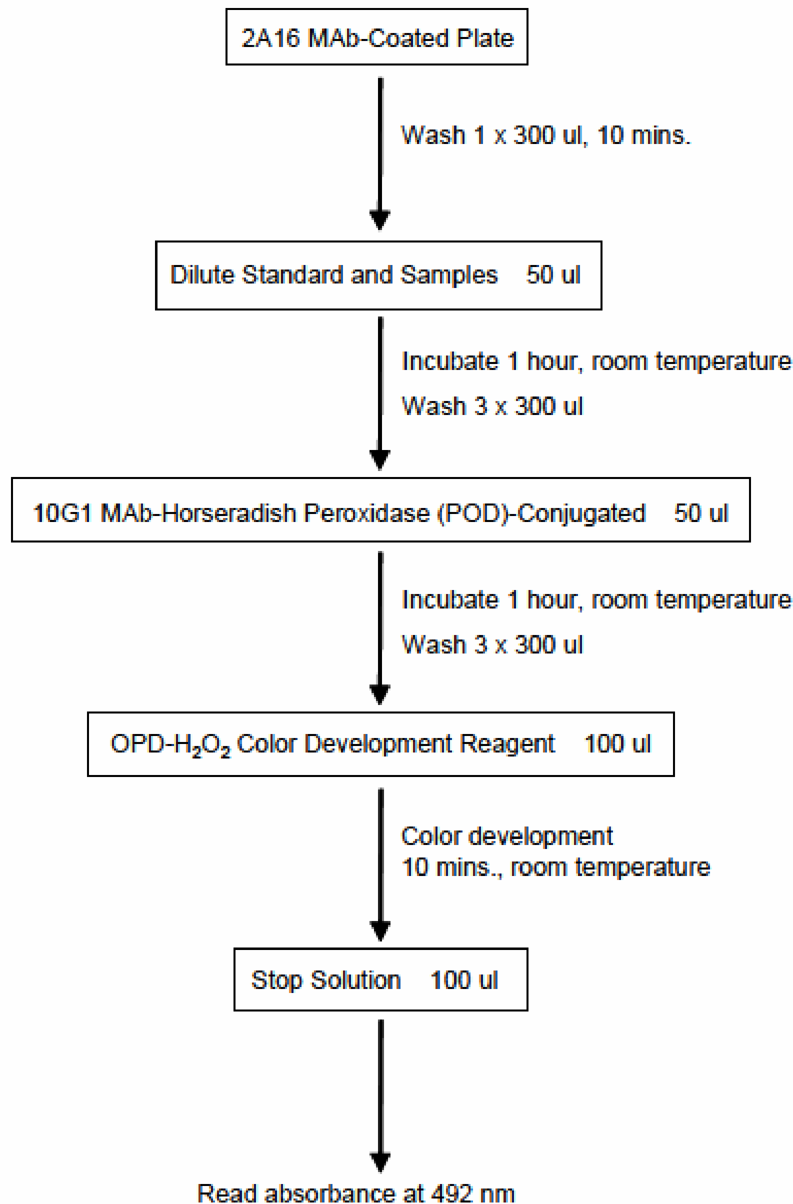
F. **Test Sample**
Plasma test samples should be diluted 1:1,600 with 1X Sample Dilution Buffer. Dilute 20 µL of test sample with 980 µL of 1X Sample Dilution Buffer for a 1:50 dilution. Mix well. Perform a second dilution by mixing 20 µL of the 1:50 test sample with 620 µL of 1X Sample Dilution Buffer for a final dilution factor of 1:1,600. Dilutions should be performed in an ice bath and kept on ice until used. The diluted samples should be used within 30 minutes of preparation.

PROCEDURE

Prepare all reagents and test samples as described above. Bring reagents to room temperature (20-30°C) prior to initiating the assay. Diluted calibrators and samples should be maintained on ice until use. To protect yourself, wear gloves when using this test kit.

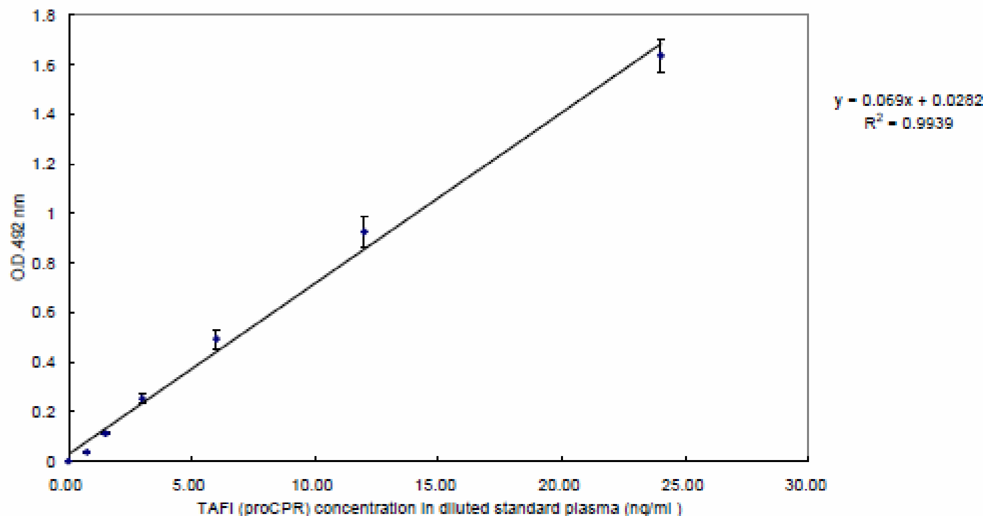
1. Remove Primary Antibody-Coated Plate from its foil pouch. Identify well position(s) for each sample on a data sheet or plate map.
2. Using a micropipette or plate washer, add 300 µL of 1X Wash Buffer to each well of the plate. The plate should be maintained at room temperature for 10 minutes.
3. Aspirate the plate to remove the wash buffer. Invert plate and gently tap on a clean absorbent towel to remove any remaining droplets.
4. Add 50 µL of diluted test samples (1:1,600) or calibrators (48, 24, 12, 6, 3, 1.5, 0.75, 0 ng/mL) to the appropriate wells.
5. Incubate the plate for 1 hour at room temperature. The plate should be covered during all incubation periods to prevent evaporation. Do not shake or agitate the plate during incubations. It is recommended that samples and calibrators are run in duplicates.
6. Aspirate the plate. Invert plate and gently tap on a clean absorbent towel to remove any remaining droplets.
7. Wash the plate 3 times with 300 µL of 1X Wash Buffer.
8. Add 50 µL of Detection Antibody Solution to each well followed by a 1 hour, room temperature incubation.

9. Aspirate the plate. Invert plate and gently tap on a clean absorbent towel.
10. Wash the plate 3 times with 300 µL of 1X Wash Buffer.
11. Aspirate the plate. Invert plate and gently tap on a clean absorbent towel.
12. After removal of the Wash Buffer, 100 µL of OPD-H_2O_2 Color Development Reagent is added to each well and incubated in the dark for 10 minutes at room temperature. Do not shake or agitate the plate.
13. Stop the reaction with 100 µL of Stop Solution in each well. Sulfuric acid is caustic. Wear protective glasses and gloves when working with the Stop Solution.
14. Determine the optical density (OD) of the plate at wavelength 492 nm. The recommended reference wavelength is 630 nm.
15. The calibration curve is prepared using the OD values of the serially diluted calibrators. The amount of TAFI in the test samples will be determined by the calibration curve.



CALCULATION OF RESULTS

1. Subtract the mean absorbance value of the 0 ng/mL blank from each mean absorbance value of the calibrator series and samples tested (Net Absorbance).
2. Plot the log of known concentrations of each calibrator and the calculated Net Absorbance on the X-axis and Y-axis, respectively. Fit an appropriate regression curve to the plotted points.
3. Determine the concentration of TAFI in the samples by interpolation of the regression curve formula.
4. Concentration calculations for the sample must be multiplied by the dilution factor to obtain the correct results for the undiluted samples.
5. If the OD >1.8 then those data points should not be used to generate the calibration curve.



TROUBLESHOOTING

1. Lack of signal or weak signal in all wells: Possible explanations: Omission of a reagent or a step, Improper preparation or storage of a reagent, Assay performed before reagents were allowed to come to room temperature, Plate reader did not perform well.
2. High signal and background in all wells: Possible explanations: Improper or inadequate washing; be certain that all wash volumes and repetitions were correct, Improper dilution of detection antibody, Overdeveloping; decrease the incubation time before the Stop Solution is added.
3. High background in sample wells only: Possible explanations: Sample concentration was too high, Improper dilution of detection antibody.
4. Weak signal in sample wells only: Possible explanations: Sample concentration was too low, Improper dilution of detection antibody.

STORAGE

Store all components at 4°C. DO NOT FREEZE.

FOR RESEARCH USE ONLY

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: techsupport@kamiyabiomedical.com
www.kamiyabiomedical.com

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