HUMAN TAFI ELISA Kit
User Manual

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See List of Components for Storage Conditions
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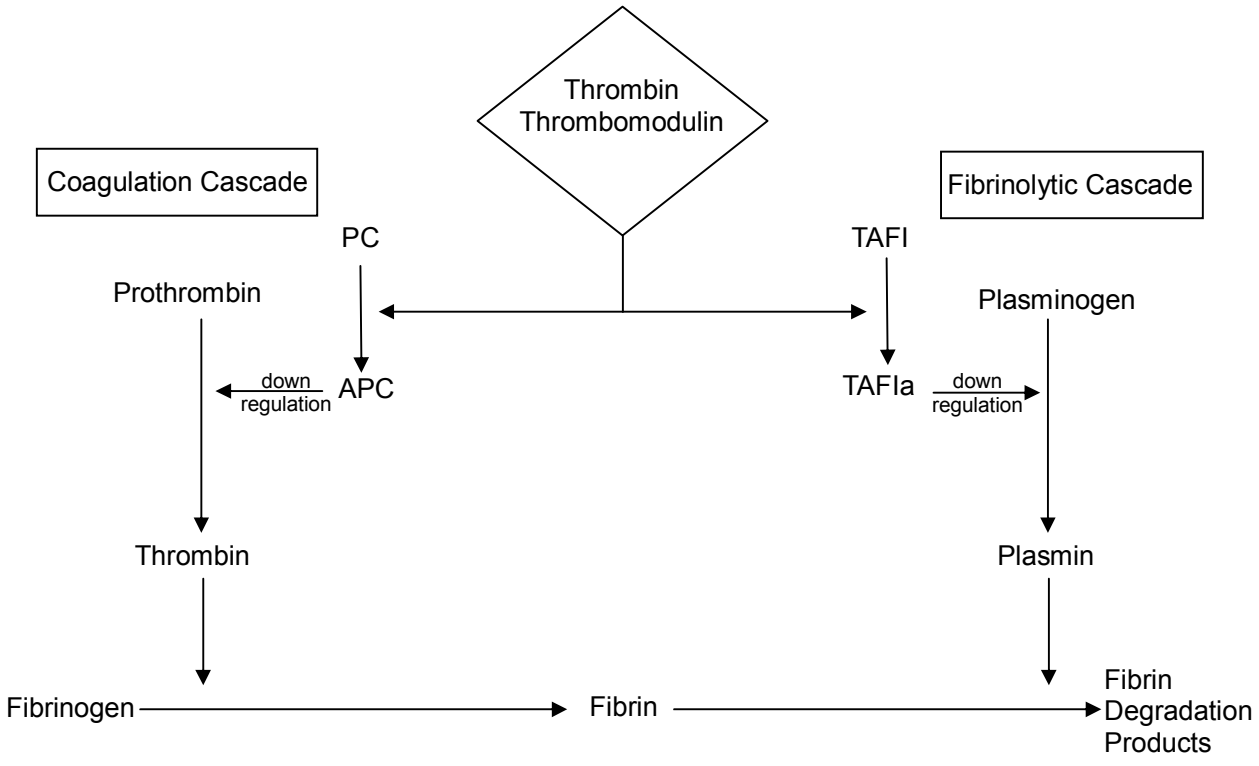
I. Introduction and Protocol Overview

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[1]. 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[2].

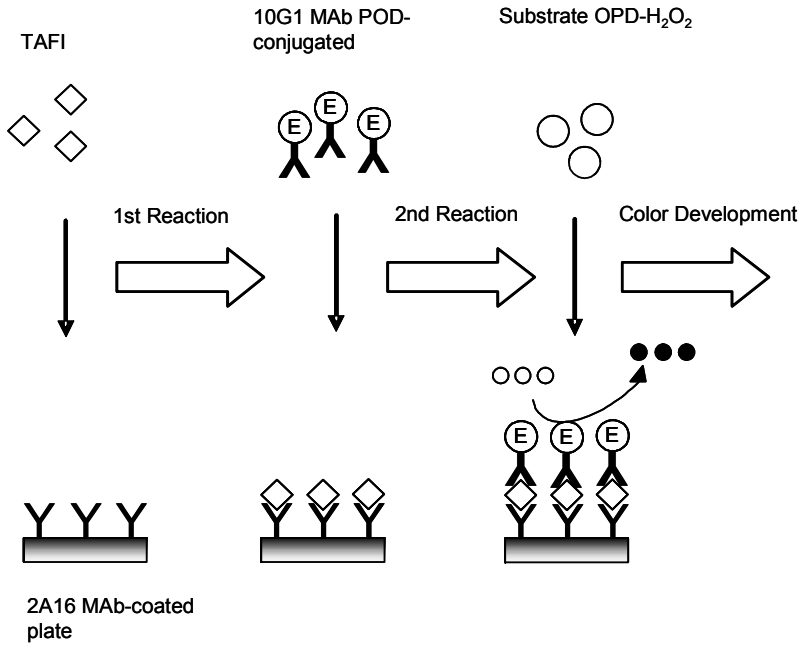
The SCETI **Human TAFI ELISA Kit** is designed to measure the concentration of TAFI in human plasma using 2 monoclonal antibodies, 2A16 and 10G1 targeted to different epitopes of TAFI[2,3].

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



The principle of the assay is shown in Figure 2. Standards and samples are incubated in a 96-well microtiter plated 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



II. List of Components

- Store all components at 2-10ºC. DO NOT FREEZE.

1	**PRIMARY ANTIBODY-COATED PLATE** One 96-well plate with adsorbed 2A16 MAb. Plate is provided in a resealable foil pouch.	1 Plate
2	**5X SAMPLE DILUTION BUFFER** 5X phosphate buffered saline with 0.5% BSA and 10 mM EDTA	1 Bottle (40 ml)
3	**6X WASH BUFFER** 6X phosphate buffered saline with 0.3% Tween 20	1 Bottle (50 ml)
4	**LYOPHILIZED STANDARD** Human plasma containing 48 ng TAFI	1 Vial
5	**DETECTION ANTIBODY** 10G1 MAb-horseradish peroxidase (POD)-conjugated	1 Vial (30 µl)
6	**30% H_2O_2 (Hydrogen Peroxide)**	1 Vial (30 µl)
7	**OPD Substrate Tablet**	1 Tablet
8	**COLOR DEVELOPING BUFFER** 33 mM citrate and 67 mM sodium phosphate (pH 5.0)	1 Bottle (20 ml)
9	**STOP SOLUTION** 2N Sulfuric acid	1 Bottle (20 ml)

III. Additional Materials Required

The following materials are required, but not supplied:

- 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 2-10ºC
- Ice bath or equivalent
- 37ºC water bath or equivalent
- Deionized water or equivalent

IV. Reagent and Standard Preparation

Buffer Dilutions

Reagents should be diluted just prior to use.

1. 1X Sample Dilution Buffer
   Prepare 1X Wash Solution by mixing 40 ml of the 5X Sample Dilution Buffer with 160 ml of deionized water or equivalent. Mix well. After preparation, store 1X Sample Dilution Buffer at 2-10°C. The 1X buffer is stable for 1 month at 2-10°C.

2. 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 deionized water or equivalent. Mix well. After preparation, the 1X Wash Buffer is stable for 1 month at 2-10°C.

Preparation of plasma standards

Add 1 ml of 1X Sample Dilution Buffer to the lyophilized standard vial to prepare 48 ng/ml TAFI. The reconstituted standard is 2-fold serial diluted in 1X Sample Dilution Buffer to prepare 24, 12, 6, 3, 1.5, and 0.75 ng/ml standards. Undiluted standard and 1X Sample Dilution Buffer will be used for 48 ng/ml and 0 ng/ml, respectively. The diluted standards will be used to generate a standard curve.

Dilutions should be performed in an ice bath and kept on ice until used. The diluted standards should be used within 30 minutes of preparation.

Preparation of 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.

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.

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

V. Sample Preparation

Plasma test samples should be diluted 1:1600 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 1:1600.

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.

VI. Human TAFI ELISA Protocol

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

Remove Primary Antibody-Coated Plate from its foil pouch. Identify well position(s) for each sample on a data sheet or plate map.

1. 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.

2. Aspirate the plate to remove the wash buffer. Invert plate and gently tap on a clean absorbent towel to remove any remaining droplets.

3. Add 50 µl of diluted test samples (1:1600) or standards (48, 24, 12, 6, 3, 1.5, 0.75, 0 ng/ml) to the appropriate wells. 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 standards are run in duplicates.

4. Aspirate the plate. Invert plate and gently tap on a clean absorbent towel to remove any remaining droplets.

5. Wash the plate 3 times with 300 µl of 1X Wash Buffer.

6. 50 µl of Detection Antibody Solution is added to each well followed by a 1 hour, room temperature incubation.

7. Aspirate the plate. Invert plate and gently tap on a clean absorbent towel.

8. Wash the plate 3 times with 300 µl of 1X Wash Buffer.

9. Aspirate the plate. Invert plate and gently tap on a clean absorbent towel.

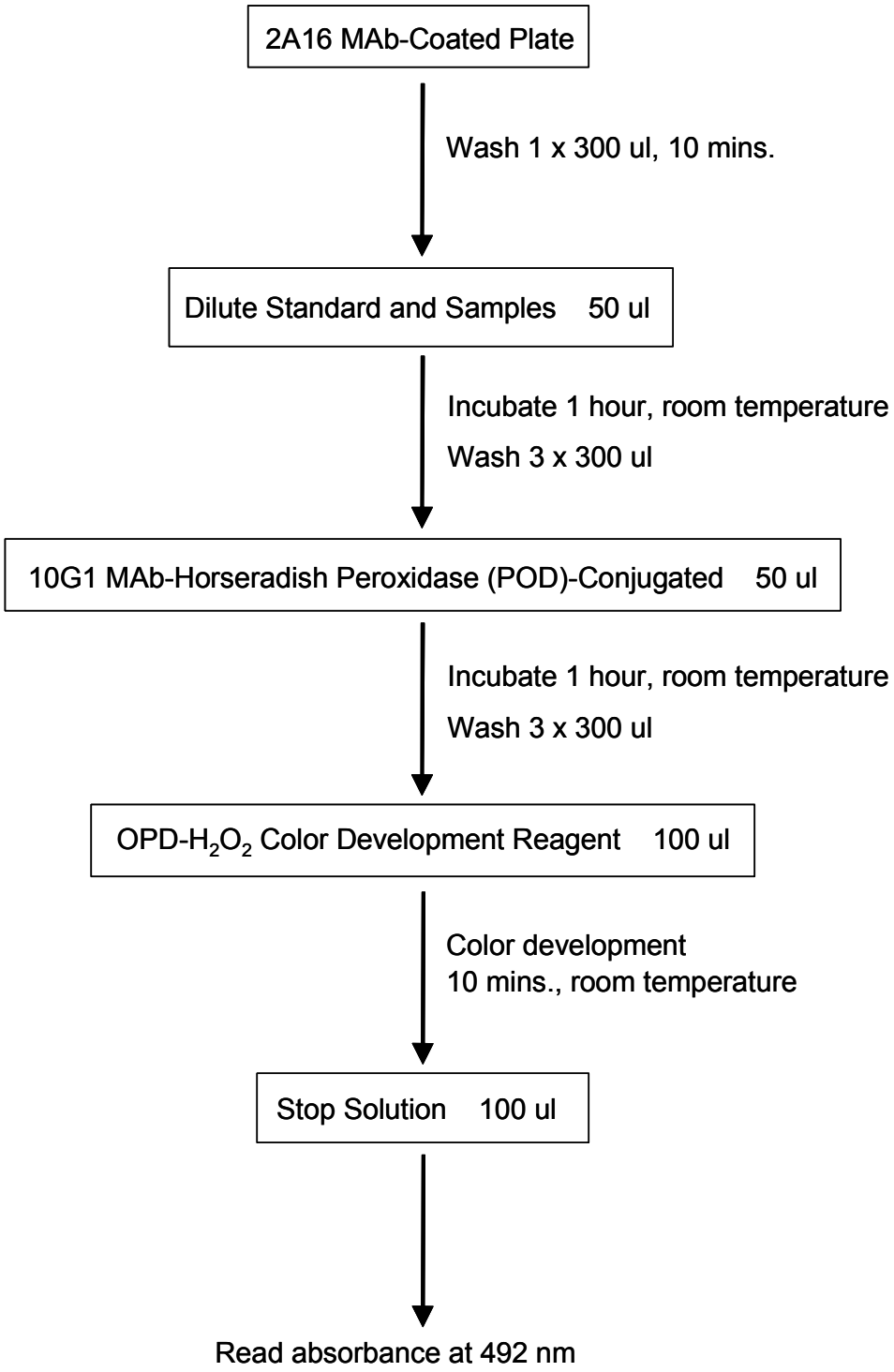
10. After removal of the Wash Buffer, 100 µl of OPD-H_2O_2 Color Development Reagent is added to each well and incubated in the dark for 10 minutes at room temperature. Do not shake or agitate the plate.

11. 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.

12. Determine the optical density (OD) of the plate at wavelength 492 nm. The recommended reference wavelength is 630 nm.

13. The standard curve is prepared using the OD values of the serially diluted standards. The amount of TAFI in the test samples will be determined by the standard curve.

Figure 3. Flow Chart of Assay Procedures



VII. Calculation of Results

1. Subtract the mean absorbance value of the 0 ng/ml blank from each mean absorbance value of the standard series and samples tested (Net Absorbance).

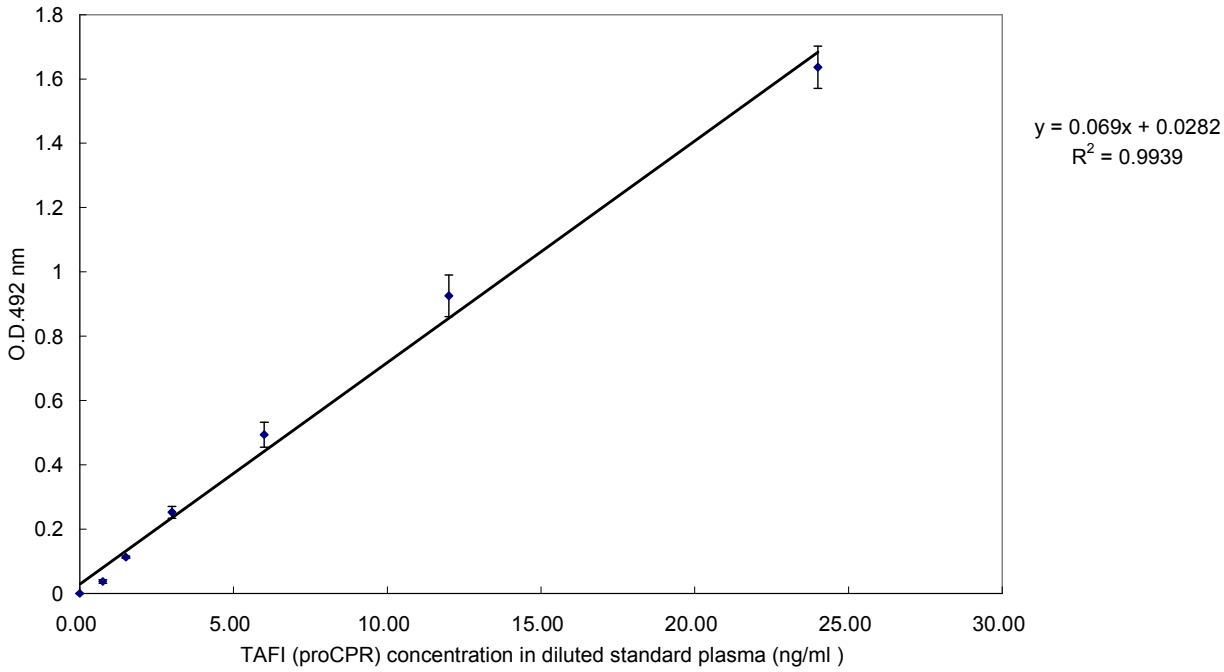
2. Plot the log of known concentrations of each standard and the calculated Net Absorbance on the X-axis and Y-axis, respectively. Fit an appropriate regression curve to the plotted points.

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 standard curve.

Figure 4. Typical Standard Curve
Plasma Standard



VIII. Troubleshooting Guide

Troubleshooting Guide

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.

IX. References

1. Nesheim, M. Thrombin and Fibrinolysis. Chest. 124:33S-39S (2003).
2. Tani, S, Akatsu, H, Ishikawa, Y, Okada, N, and Okada, H. Preferential detection of procarboxypeptidase R by enzyme-linked immunosorbent assay. Microbiol. Immunol. 47:295-300 (2003).
3. Guo, YY, Morioka, A, Kaneko, Y, Okada, N, Obata, K, Nomura, T, Campbell, W, and Okada, H. Arginine carboxypeptidase (CPR) in human plasma determined with sandwich ELISA. Microbiol. Immunol. 43:691-698 (1999).

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