Affinity Biologicals™ INC.

REPRESENTATIVE DATA SHEET

VisuLize™ TAFI Antigen Kit

96 Test Enzyme Immunoassay Kit for Thrombin Activatable Fibrinolysis Inhibitor (TAFI, proCPU) antigen

For Research Use Only.
Not for use in diagnostic procedures.

Product # TAFI-AG

Store at 2–8° C. Do Not Freeze.

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INTENDED USE

The *VisuLize*™ TAFI Antigen kit is an Enzyme Immunoassay for the quantitative determination of TAFI antigen in plasma samples using an enzyme linked immuno-sorbant assay (ELISA).

SUMMARY

TAFI (Thrombin Activatable Fibrinolysis Inhibitor), also referred to as plasma procarboxypeptidase-B, procarboxypeptidase-U and R, circulates in plasma as a zymogen with a mass of 58,000 daltons (1-7). Proteolytic activation of TAFI yields an N-terminally derived activation peptide and the C-terminal portion corresponding to the metalloprotease, activated TAFI (TAFIa). TAFIa exhibits exopeptidase activity with carboxypeptidase B-like substrate specificity capable of catalyzing the hydrolysis of C-terminal lysine and arginine residues. Cleavage of these residues on fibrin by TAFIa attenuates clot lysis by inhibiting the formation of the ternary activation complex comprising fibrin cofactor, tPA and plasminogen, thereby inhibiting plasmin generation. Although TAFI can be activated by various proteases including thrombin and plasmin, the physiological activator is proposed to be the complex thrombin-thrombomodulin since the rate of activation is stimulated 1250-fold compared to thrombin alone (4). However, the rate of TAFI activation is highly dependent upon its plasma concentration. Since TAFIa apparently plays a key role in connecting coagulation and fibrinolysis and significantly increases clot stability, determination of plasma concentration of TAFI is likely crucial to assess its subsequent potential antifibrinolytic effects. This has been demonstrated in a report identifying high plasma concentration of TAFI as a risk factor for thrombosis (7).

PRINCIPLE

Strip wells are pre-coated with polyclonal antibody to human TAFI. Plasma samples are diluted and applied to the wells. The TAFI present binds to the coated antibody. After washing away unbound material, peroxidase-labeled detecting antibody is applied and allowed to bind to the captured TAFI. The wells are again washed and a solution of tetramethylbenzidine (TMB, a peroxidase substrate) is applied and allowed to react for a fixed period of time. A blue color develops which changes to yellow upon quenching the reaction with acid. The color formed is measured spectrophotometrically in a microplate reader at 450 nm. The absorbance at 450 nm is proportional to the quantity of TAFI captured onto the well. The assay is calibrated using the reference plasma provided in the kit.

REAGENTS

A. Description of Reagent Items

Item 1: Foil pouch containing 6 strips, each containing 16 wells coated with sheep antibody to human TAFI.
Item 2: 2 vials of Standard Reference Plasma, each lyophilized from 1 ml plasma.
Item 3: 2 vials of Control Plasma A, each lyophilized from 1 ml plasma.
Item 4: 2 vials of Control Plasma B, each lyophilized from 1 ml plasma.
Item 5: 2 vials of TAFI Deficient Plasma, each lyophilized from 1 ml deficient plasma.
Item 6: 1 vial containing 30 ml of a 10X Wash Buffer Concentrate.
Item 7: 3 vials, each containing 20 ml of buffered Sample Diluent.
Item 8: 1 vial of 12 ml peroxidase-labeled detecting antibody.
Item 9: 1 vial of 12 ml of tetramethylbenzidine (TMB) substrate.
Item 10: 1 vial of 12 ml Stop Solution containing 0.2 M Sulphuric acid.

B. Reagent Preparation

Item 1 (Antibody-coated stripwell plate): Just prior to use open pouch and remove strips and frame. Unused strips should be replaced in the pouch and resealed. Strips must be washed before use, see section C: Assay Procedure.
Item 2 (Standard Reference plasma): Reconstitute one vial with 1.0 ml of reagent grade water. Allow contents to dissolve for 15 minutes at room temperature with occasional swirling. Stability after reconstitution is 8 hours at 2-8°C, or 1 month at –20°C.
Items 3 and 4 (Control plasmas A and B): Reconstitute one vial of each plasma with 1.0 ml of reagent grade water. Allow contents to dissolve for 15 minutes at room temperature with occasional swirling. Stability after reconstitution is 8 hours at 2-8°C, or 1 month at –20°C.
Item 5 (TAFI Deficient Plasma): Reconstitute one vial with 1.0 ml of reagent grade water. Allow contents to dissolve for 15 minutes at room temperature with occasional swirling. Stability after reconstitution is 8 hours at 2-8°C, or 1 month at –20° C.
Item 6 (Wash Buffer Concentrate): Allow vial to warm to room temperature before use. Ensure any crystals that may have formed are dissolved before proceeding. If necessary the vial can be warmed to 37°C until all crystals have dissolved. Dilute the concentrate by adding 30 ml concentrate to 270 ml reagent grade water and mix. Stability after dilution is 2 days at 2–8°C.

Remaining component items are supplied ready to use.

C. Storage and Stability

Intact kits and un-reconstituted reagents are stable until the expiration date stated on the box and individual reagent labels when stored at 2–8°C.

D. Caution and Warning

For Research use only. Not for use in Diagnostic procedures.
Some items contain human source material. Each unit of source plasma used in the preparation of this product has been tested by an FDA approved method and found negative for the presence of Human Immunodeficiency Virus (HIV) Type I and Type II, Hepatitis B surface antigen (HB_SAg) as well as for Hepatitis C (HCV). However, no test can offer complete assurance that products derived from human blood will not transmit infectious diseases. As with all materials of human origin, this product should be handled as a potentially infectious material.
The substrate TMB (tetramethylbenzidine) has reduced toxicity, but precautions should still be taken to avoid direct contact. The use of gloves and safety glasses are recommended.
The Stop Solution contains dilute sulphuric acid (0.2 M), which is corrosive. The use of gloves and safety glasses are recommended.

SPECIMEN COLLECTION

Blood is collected into 3.2% Buffered Citrate anticoagulant tubes at a ratio of 9 volumes blood to 1 volume anticoagulant and gently mixed by inversion. Centrifuge at a minimum of 1500 x g for 10 minutes. Remove supernatant plasma and use within 4 hours or freeze below –20°C for 1 month.

PROCEDURE

A. Material Provided

Foil pouch containing 6 strips of antibody coated wells.
Standard Reference Plasma, lyophilized.
Control Plasma A, lyophilized.
Control Plasma B, lyophilized.
TAFI Deficient Plasma, lyophilized.
10X Wash Buffer Concentrate.
Sample Diluent.
Detecting antibody solution.
TMB substrate.
Stop Solution.
Adhesive Plate Sealer.

B. Additional Material Required (but not provided)

Reagent grade water for reconstitution and for dilution
Single-channel adjustable volume pipettes
Eight-channel pipettes
Laboratory timer
Microplate strip-well washer device
Microplate compatible spectrophotometer capable of 450 nm.

C. Assay Procedure

Reconstitute reagents as described in REAGENTS. Allow reagents to warm to room temperature before use.
NOTE: It is recommended that all standard, controls and test dilutions be run in duplicate and that each run include a buffer blank (see Assay Calibration section).

1. **Prepare all sample dilutions immediately prior to use on plate.**
2. **Preparation of Standard Reference Plasma:** Dilute the Standard Reference Plasma (reconstituted Item 2) into TAFI Deficient plasma (reconstituted Item 5) as detailed in Table 1 below:

TABLE 1:

Dilution	Std Reference Plasma	TAFI Deficient Plasma
Neat	30 µl	---
1/2	30 µl	30 µl
1/4	30 µl of 1/2 dilution	30 µl
1/8	30 µl of 1/4 dilution	30 µl
1/16	30 µl of 1/8 dilution	30 µl
1/32	30 µl of 1/16 dilution	30 µl

Make a further 1/100 dilution of each of the diluted samples from Table 1 by adding 10 µl plasma to 0.99 ml of Sample Diluent (Item 7). **Ensure sample diluent is warmed to room temperature before use. Ensure any crystals that may have formed are dissolved before proceeding.** The final dilutions for the standard curve will be 1/100 (from the "Neat" plasma), 1/200, 1/400, 1/800, 1/1600 and 1/3200, with the 1/100 dilution corresponding to the reference value stated on the Standard Reference Plasma label.

3. **Control and Sample Preparation:** The 2 Control plasmas (reconstituted Items 3 and 4) and test plasmas are first diluted 1/2 in TAFI deficient plasma and then further diluted 1/100 in Sample Diluent to obtain a final dilution of 1/200.
4. **Assay:**

PLATE PREPARATION	Place desired number of strips into frame. Before use wash strips with at least 300 µl per well of diluted Wash Buffer (Item 6). Empty wells and repeat twice for a total of three washes.	
STEP	Pipette into each pre-coated well:	
TAFI CAPTURE	Test Sample (run in duplicate)	100 µl
	Cover strips with the plate sealer and incubate 1 hour at ambient temperature.	
Empty wells and wash with diluted wash buffer 3 times.		
DETECTING ANTIBODY	Detecting Antibody Solution (Item 8)	100 µl
	Cover strips with the plate sealer and incubate 30 minutes at ambient temperature.	
Empty wells and wash with diluted wash buffer 3 times.		
COLOR DEVELOPMENT	TMB Substrate (Item 9)	100 µl
	Allow color to develop for **exactly** 10 minutes at ambient temperature.	
	Stop Solution (Item 10)	100 µl (Add to each well in same order in which the TMB was added)
Read plate at a wavelength of 450 nm within 30 minutes of adding Stop Solution		

CALIBRATION

A. Assay Calibration

The TAFI value stated on the Standard Reference Plasma vial has been assayed against a reference plasma traceable to purified TAFI.
It is recommended that all standards and tests be performed in duplicate.
It is recommended that the plate be blanked on wells that have received Sample Diluent alone instead of diluted sample (reagent blank wells).

B. Reference Curve

The reference curve is constructed manually by plotting the mean absorbance values (y axis) versus the TAFI concentration (x axis) on log-log graph paper. Alternatively, curve-fitting software may be used to obtain a reference curve using log-log or a 4-parameter algorithm.

QUALITY CONTROL

The supplied Control Plasmas (Item 3 and 4) should be assayed with every series of samples that are run. The TAFI values obtained for the samples are only valid if the values obtained for the control plasmas are within the range stated on the Control Plasma labels.

EXPECTED RESULTS

The TAFI content of test samples and controls can be read off the reference curve and multiplied by the appropriate dilution factor. Under the above conditions, a sample diluted 1/100 will have a dilution factor of 1 whereas a sample diluted 1/200 will have a dilution factor of 2.

Example: A test plasma when diluted 1/200 gives an absorbance corresponding to 5.4 µg/ml when read from the reference curve. This value would appropriately be multiplied by a dilution factor of 2 to obtain the corrected value of 10.8 µg/ml.

LIMITATIONS AND INTERFERENCES

Results from 3 lots demonstrated no interference by Rheumatoid Factor. The theoretical possibility of anti-sheep antibodies in test samples would cause erroneous results. There may be some interference from therapeutic agents such as standard heparin, ε-amino-n-caproic acid or tranexamic acid. Further studies are required to determine the possible effect of these substances in this assay.

EXPECTED VALUES

Each laboratory should determine a normal range independently, but results from 3 lots indicate a normal range of 5.8 to 10.0 µg/ml (100 to 172 nM).

PERFORMANCE CHARACTERISTICS

A. Reactivity

This assay measures total TAFI antigen in plasma.

B. Detection Limit and Working Range

The limit of detection is 3.13% of the standard reference value. For example, if the Standard Reference Plasma has a TAFI level of 6.5 ug/ml, the limit of detection for the assay would be 6.5 x 0.0313 = 0.2 ug/ml. Plasma samples containing less than 0.5 ug/ml should be repeated at a 1/100 dilution in Sample Diluent. When purified preparations of TAFI are being assayed it is recommended that an initial dilution be made in TAFI-deficient plasma to an estimated concentration of 5 ug/ml. Further dilutions should then be prepared in a manner similar to that of the Standard Reference Plasma and Controls i.e. 1/2, 1/4, 1/8 etc. dilution in TAFI deficient plasma followed by a further 1/100 dilution in Sample Diluent.

C. Precision

Maximum variability calculated from 3 lots: Intra-Assay C.V. = 5.8%; Inter-Assay C.V. = 8.0%.

D. Lot-to-Lot Variability

Control plasma values determined in 3 different lots indicated a lot-to-lot variability of 6.2%.

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