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VISULIZE™ TAFI Antigen ELISA Kit

CL20126E
Lot :
Exp:

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).

Principle:
Strip wells are pre-coated with polyclonal antibody to human TAFI. Plasma samples are diluted and applied to wells. The TAFI present binds to the coated antibody. After washing away unbound material, peroxidase-labelled 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 colour develops which changes to yellow upon quenching the reaction with acid. The colour 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.

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Materials Supplied

A. Description of Reagent Items

Catalog No.	Component	Quantity
CL20126E-A	Foil pouch containing 6 strips, each containing 16 wells coated with sheep antibody to human TAFI ***(see "Reagent Preparation")***	6 strips, 16 wells Each
CL20126E-A2	Plate sealer	1
CL20126E-B	Standard Reference Plasma (2) each lyophilized from 1 ml plasma ***(see "Reagent Preparation")***	2
CL20126E-C	Control Plasma A (2), each lyophilized from 1 ml plasma ***(see "Reagent Preparation")***	2
CL20126E-D	Control Plasma B (2), each lyophilized from 1 ml plasma ***(see "Reagent Preparation")***	2
CL20126E-E	TAFI Deficient Plasma (2), each lyophilized from 1 ml deficient plasma ***(see "Reagent Preparation")***	2
CL20126E-F	10X Wash Buffer Concentrate. ***(see "Reagent Preparation")***	30 ml
CL20126E-G	Buffered Sample Diluent (3), ready to use	3 x 20 ml
CL20126E-H	Peroxidase-labeled detecting antibody, ready to use	12 ml
CL20126E-I	Tetramethylbenzidine (TMB) substrate, ready to use	12 ml
CL20126E-J	Stop Solution containing 0.2 M Sulphuric acid, ready to use	12 ml

B. Reagent Preparation

Antibody-coated stripwell plate (CL20126E-A): 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: ***Assay Procedure.***

Standard Reference plasma (CL20126E-B): 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 +4ºC, or 1 month at –20ºC.

Control plasmas A (CL20126E-C) and B (CL20126E-D): 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 + 4ºC, or 1 month at –20ºC

TAFI Deficient Plasma (CL20126E-E): 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 +4ºC, or 1 month at –20ºC.

Wash Buffer Concentrate (CL20126E-F): 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 37ºC until the 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 + 4ºC. Remaining component items are supplied ready to use.

C. Storage

Intact kits and un-reconstituted reagents are stable until the expiration date stated on the box and individual reagent labels when stored at +4ºC. **Do Not Freeze**.

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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 preparation of this product has been tested by the FDA approved method and found negative for the presence of Human Immunodeficiency Virus (HIV) Type I and Type II, Hepatitis B surface antigen (HbsAg) 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 –20ºC for 1 month.

E. Additional Materials 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.

F. Assay Procedure

Reconstitute reagents as described in ***Reagent Preparation***. 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 CL20126E-B) into TAFI deficient plasma (reconstituted CL20126E-E) 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

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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 (CL20126E-G). **Ensure sample diluent is warmed to room temperature before use. Ensure 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, 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 CL20126E-C and CL20126E-D) and test plasmas are first diluted 1/2 in TAFI deficient plasma and then further diluted 1/100 Sample Diluent to obtain a final dilution of 1/200.

4. Assay:

<table>
<tr><td>PLATE PREPARATION</td><td colspan="2">Place desired number of strips into frame. Before use wash strips with at least 300 µl per well of diluted Wash Buffer (CL20126E-F). Empty wells and repeat twice for a total of three washes.</td></tr>
<tr><td>STEP</td><td colspan="2">Pipette into each pre-coated well:</td></tr>
<tr><td rowspan="2">TAFI CAPTURE</td><td>Test Sample
(run in duplicate)</td><td>100 µl</td></tr>
<tr><td colspan="2">Cover strips with the plate sealer and incubate 1 hour at ambient temperature</td></tr>
<tr><td colspan="3">Empty wells and wash with diluted wash buffer 3 times.</td></tr>
<tr><td rowspan="2">DETECTING ANTIBODY</td><td>Detection Antibody Solution
(CL20126E-H)</td><td>100 µl</td></tr>
<tr><td colspan="2">Cover strips with the plate sealer and incubate 30 minutes at ambient temperature</td></tr>
<tr><td colspan="3">Empty wells and wash with diluted wash buffer 3 times</td></tr>
<tr><td rowspan="3">COLOUR DEVELOPMENT</td><td>TMB Substrate
(CL20126E-I)</td><td>100 µl</td></tr>
<tr><td colspan="2">Allow colour to develop for <u>exactly 10 minutes</u> at ambient temperature</td></tr>
<tr><td>Stop Solution
(CL20126E-J)</td><td>100 µl
(Add to each well in same order in which the TMB was added)</td></tr>
<tr><td colspan="3">Read plate at a wavelength of 450 nm within 30 minutes of adding Stop Solution</td></tr>
</table>

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 preformed 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.

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Quality Control

The supplied Control Plasmas (CL20126E-C and CL20126E-D) 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 in 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. Theoretically, if antisheep antibodies were present in human test samples there would be 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 µg/ml, the limit of detection for the assay would be 6.5 x 0.0313 = 0.2 µg/ml. Plasma samples containing less than 0.5 µg/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 µg/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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Laboratory Reagent For Research Use Only

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