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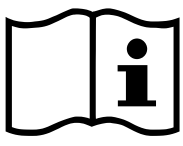
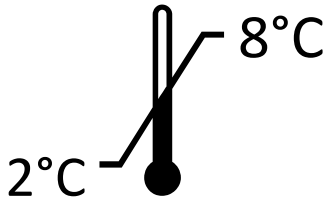
# IMUCLONE™ Total TAFI ELISA

## REF 873

*For Research Use Only*



## INTENDED USE

The IMUCLONE™ Total TAFI ELISA is an *in vitro* assay for the measurement of TAFI antigen in human plasma, or in any fluid where TAFI may be present.

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

## EXPLANATION OF THE TEST

TAFI, Thrombin Activatable Fibrinolytic Inhibitor, (also known as carboxypeptidase U and plasma pro-carboxypeptidase B) is a 60,000 D molecular weight glycoprotein (proenzyme form) present in human plasma[1] that modulates fibrinolysis in vivo. This proenzyme is converted to a 35,000 D molecular ratio active form, TAFIa, following proteolytic cleavage by the thrombin/thrombomodulin complex. TAFIa possesses carboxypeptidase activity with a preference for cleaving lysine and arginine residues from the c-terminus of proteins. Modulation of fibrinolysis occurs when TAFIa cleaves C-terminal arginine and lysine residues of partially degraded fibrin.[2,4,5] The removal of the c-terminus arginine and lysine residues from fibrin inhibits the continued degradation of fibrin by tPA activated plasmin.[3]

Plasma also contains carboxypeptidase N (CPN), which has enzymatic activity similar to that of TAFI. The total carboxypeptidase activity in plasma is found to be TAFI + CPN. TAFI, but not CPN, is inhibited by potato tuber carboxypeptidase inhibitor (PTCI). The ability of PTCI to selectively inhibit TAFI is used to determine that portion of the total carboxypeptidase activity contributed by TAFI.

TAFI may play a central role in thrombosis and fibrinolysis due to its ability to retard fibrin clot lysis[3].

## PRINCIPLE OF THE PROCEDURE

Diluted plasma samples, biological fluid or TAFI Calibrator are added to microwells precoated with a murine monoclonal antibody specific for human TAFI. The antibody captures the TAFI antigen present in the solutions during an incubation period. Following a washing step, a goat anti-human TAFI polyclonal antibody coupled to horseradish peroxidase (HRP) is added to the microwells and binds to the captured TAFI antigen. Following another washing step, the peroxidase substrate Tetramethylbenzidine (TMB), in the presence of hydrogen peroxide, is added to the wells and the subsequent reaction yields a blue colored solution. Addition of sulfuric acid stops the reaction and turns the solution color yellow. The absorbance of the solution is measured at 450 nm. The absorbance is directly proportional to the amount of TAFI present in the sample.

## REAGENTS

96-Well Microtest Plate pre-coated with anti-Human Total TAFI; 12 strips, 8 wells/strip in a frame holder plus storage bag with desiccant
2 vials of Sample Diluent-F, ready to use (50 mL)
3 vials of Plasma TAFI Calibrator (lyophilized)
1 vial TAFI Control I - High (lyophilized)
1 vial TAFI Control II - Low (lyophilized)
1 vial of Conjugate Diluent, ready to use (25 mL)
3 vials of Anti-HumanTotal-TAFI-HRP Immunoconjugate (lyophilized)
1 vial Wash Solution, 20 fold concentrate (50 mL)
1 vial of TMB Substrate (Peroxidase Substrate), ready to use (25 mL)
1 vial of Stop Solution (0.45 M Sulfuric Acid), ready to use (6 mL)

## WARNINGS AND PRECAUTIONS

Source material for some of the reagents in this kit is of human origin. This material has been found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV-2). As no known test method provides complete assurance that products derived from human blood will not transmit HBsAg, HCV, HIV-1, HIV-2 or other blood-borne pathogens, reagents should be handled as recommended for any potentially infectious human specimen. Discard all waste associated with test specimens and human source reagents in a biohazard waste container.

For Research Use Only. Not for internal use in humans or animals. Do not use the kit components beyond the stated expiration date. Do not mix reagents from different kits. Avoid microbial contamination of the reagents. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled. Do not pipette reagents by mouth. Wear laboratory coat and disposable gloves throughout the test procedure and wash hands thoroughly afterwards. Avoid splashing or aerosol formation.

| | | |
|---|---|---|
| Conjugate Diluent | Warning  | CONT: Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1)<br>H317, P261, P272, P280, P302+P352, P333+P313, P363, P501 |
| Sample Diluent-F | Warning  | CONT: Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1)<br>H317, P261, P272, P280, P302+P352, P333+P313, P363, P501 |
| Wash Solution | Warning  | CONT: Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1)<br>H317, P261, P272, P280, P302+P352, P333+P313, P363, P501 |
| Stop Solution | Danger  | CONT: Sulfuric acid<br>H290, P234, P390 |

| | | |
|---|---|---|
| **Hazard Statements:** | H290 | May be corrosive to metals. |
| | H317 | May cause an allergic skin reaction. |
| **Precautionary Statements:** | P234 | Keep only in original container. |
| | P261 | Avoid breathing dust/fume/gas/mist/vapors/spray. |
| | P272 | Contaminated work clothing should not be allowed out of the workplace. |
| | P280 | Wear protective gloves/ protective clothing/ eye protection/ face protection. |
| | P302 + P352 | IF ON SKIN: Wash with plenty of soap water. |
| | P333 + P313 | If skin irritation or rash occurs: Get medical advice/attention. |
| | P363 | Wash contaminated clothing before reuse. |
| | P390 | Absorb spillage to prevent material damage. |
| | P501 | Dispose of contents/container in consultation with your regional waste disposer |

## REAGENT PREPARATION AND STORAGE

Unopened and lyophilized reagents are stable until the expiration date printed on the box when properly stored at 2°-8°C. Allow the reagents to warm to room temperature for 30 minutes before use.

1. **Microwell Strips pre-coated with anti-Human Total TAFI:** Once removed from the aluminium pouch, the microwell strips must be used within 30 minutes. Unused strips may be stored at 2°-8°C for 4 weeks when sealed in the original pouch with the desiccant present, protected from any moisture, and stored in the provided storage bag.
2. **Sample Diluent-F:** Supplied ready to use, once opened, the diluent may be used for up to 4 weeks when stored at 2°-8°C.
3. **Plasma TAFI Calibrator:** Reconstitute each vial with 0.5 mL of distilled water. Reconstituted calibrator is stable for at least 8 hours at 18°-25°C and 24 hours at 2°-8°C.
4. **TAFI Controls I and II:** Reconstitute each vial with 0.5 mL of distilled water. The controls are stable for 8 hours at room temperature, 24 hours at 2°-8°C or for 2 months at –20°C. Concentrations and acceptance ranges are indicated on the accompanying flyer.
5. **Conjugate Diluent:** It is ready to use. Once opened, it may be used for up to 4 weeks when stored at 2°-8°C.
6. **Anti-Human Total TAFI-HRP Immunoconjugate:** Reconstitute each vial with 7.5 mL of Conjugate Diluent. Shake the vial gently to homogenize the content. Reconstituted conjugate is stable for at least 24 hours at room temperature or for up to 4 weeks at 2°-8°C.
7. **Wash Solution:** If solids are present, incubate the vial for 15-30 minutes in a 37°C water bath. Shake the vial and dilute the amount required 1:20 in distilled water (the entire vial is sufficient to prepare 1 Liter of Wash Solution). The Wash Solution may be used for up to 4

weeks after opening when stored at 2°-8°C in its original vial. Diluted Wash Solution may be used for up to 7 days when stored at 2°-8°C.

8. **TMB Substrate:** It is ready to use. Once opened, it may be used for up to 4 weeks when stored at 2°-8°C.
9. **Stop Solution (0.45 M Sulfuric Acid):** It is ready to use. Warning: Sulfuric acid is caustic. Handle with care. Avoid any skin and eye contact. Wear protective glasses and gloves when handling.

## SPECIMEN COLLECTION AND PREPARATION

Either citrate or EDTA collected platelet poor plasma may be used for this assay. Plasma collection should be performed as follows:

1. Collect 9 parts of blood into 1 part of 3.2% (0.109M) trisodium citrate anticoagulant solution.
2. Centrifuge the blood sample at 2,500 x g for 20 minutes.
3. Plasma should be stored at 2°-8°C and assayed within 8 hours. Alternatively, plasma may be stored at –20°C or colder for up to 6 months.
4. Frozen plasma should be thawed rapidly at 37°C. Thawed plasmas should be stored at 2°-8°C and assayed within 4 hours.

The TAFI Controls and test sample must be tested diluted 1:50 in the Sample Diluent-F. For expected TAFI concentrations >100%, samples may be assayed at a higher dilution, 1:100, 1:200 or higher.

## PROCEDURE

**Materials Provided** – See Reagents

### Materials Required But Not Provided

Distilled water
50-300 µL eight channel multi-pipette
0-200 µL, 200-1000 µL single pipettes
Microwell plate reader for reading absorbance at 450 nm
Microwell plate washer (optional)

### Preparation of the TAFI Ag Concentrations

TAFI Ag concentrations are expressed as % of pooled normal plasma (which concentration is assigned to 100%). For the TAFI Ag assay the 100% concentration corresponds to a normal pool human plasma diluted 1:50, which is the standard assay dilution.

Dilute the reconstituted Plasma TAFI Calibrator 1:50 with Sample Diluent-F. Using this diluted calibrator, with the TAFI Ag concentration "C" indicated on the enclosed flyer, prepare the following TAFI Ag concentrations:

| TAFI Ag concentration (%) | C | C/2 | C/4 | C/10 | C/20 | 0 |
|---|---|---|---|---|---|---|
| **Vol. of 1:50 diluted TAFI Calibrator** | 1 mL | 0.5 mL | 0.25 mL | 0.1 mL | 0.05 mL | 0 mL |
| **Vol. of Sample Diluent-F** | 0 mL | 0.5 mL | 0.75 mL | 0.9 mL | 0.95 mL | 1 mL |

Mix each TAFI Ag concentration gently to ensure complete mixing. The TAFI Ag concentrations are stable for at least 4 hours at room temperature.

Remove the required number of microwell strips from the aluminium pouch sufficient for the number of assays to be performed. Place strips in the frame provided. To the appropriate wells, add reagents and perform the various assay steps as indicated on the following table:

| Reagent | Volume | Procedure |
|---|---|---|
| TAFI Ag concentration, diluted control, or diluted test sample | 200 µL | Add the TAFI Ag concentration, diluted controls or diluted test samples to appropriate microwell. |
| Incubate for 2 hours at 37°C | | |
| Wash Solution | 300 µL | Wash the wells 5 times. |
| Anti-Human Total TAFI-HRP Immunoconjugate | 200 µL | Add the immunoconjugate to each microwell. |
| Incubate for 1 hour at 37°C | | |
| Wash Solution | 300 µL | Wash the wells 5 times. |
| TMB Substrate | 200 µL | Add the substrate immediately after washing the wells. |
| Incubate for exactly 5 minutes at room temperature (18-25°C) | | |
| Stop Solution (0.45 M $H_2SO_4$) | 50 µL | Following exactly the same time intervals used for adding the substrate, stop the reaction by adding 0.45M $H_2SO_4$. |
| Wait for 10 minutes to allow the color to stabilize and measure the absorbance at 450 nm. | | |

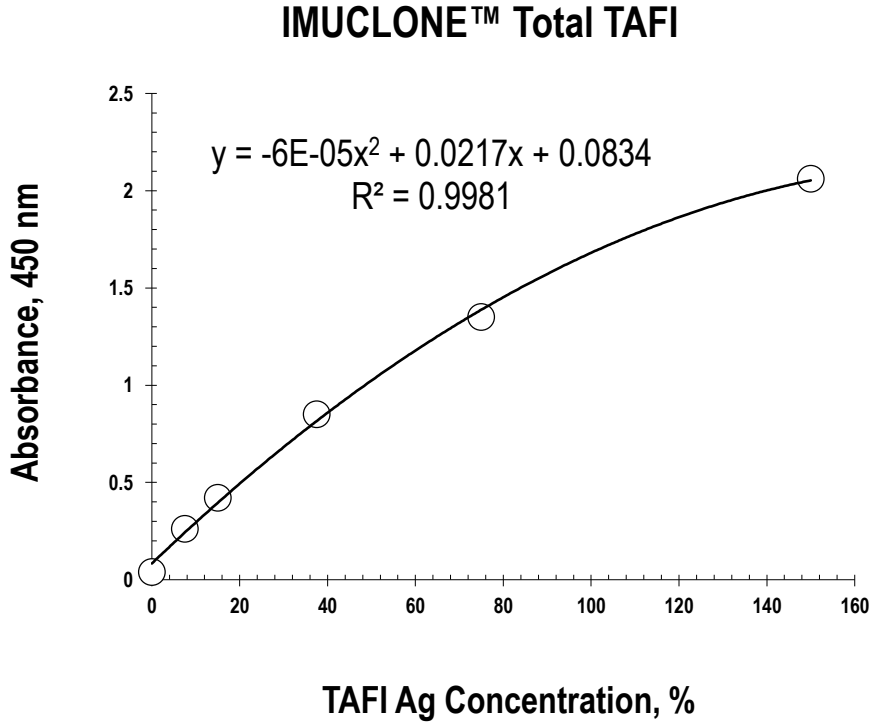
Notes:

1. Avoid leaving the plate in bright sunlight during incubations and particularly during color development.

2. Do not allow the microwells to dry out between the addition of reagents or following a washing step. Add the next reagent within 3 minutes to prevent the microwells from drying, which could damage the immobilized components. If necessary, fill the microwells with prepared Wash Solution and empty them just before the introduction of the next reagent.
3. When adding the TMB Substrate, the time interval between each row must be accurate and exactly determined. It must be the same when stopping the reaction.
4. For bichromatic absorbance readings, a reference wavelength of 690 nm or 620 nm may be used.

## RESULTS

Construct a calibration curve by plotting the mean absorbance value for each TAFI Ag concentration versus the corresponding TAFI Ag concentration (%). A calibration curve should be generated each time the assay is performed. The following calibration curve is for demonstration purposes only.




## CALCULATIONS

From the calibration curve obtained, directly interpolate the TAFI Ag concentration in samples tested at the standard 1:50 dilution. If a higher dilution was used, the TAFI Ag concentration must be multiplied by the complementary dilution factor, i.e. if the sample was diluted by 1:100, multiply the measured concentration result by 2. Alternatively, an ELISA software (i.e. Dynex, etc.) can be used for the calculation of concentrations.

## LIMITATIONS OF THE PROCEDURE

There are no known limitations for the assay.

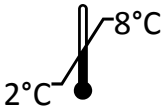
## EXPECTED VALUES

The TAFI antigen concentration in normal human plasma is usually between 40 and 250%[1].

## REFERENCES


1. Chetaille, P., et al. *Thrombosis and Haemo*stais 2000, **83**: 902-905.
2. Boffa, M. B., et al. *Journal of Biological Chemistry* 1998, **273**: 2127-2135.
3. Mosnier, L. O., et al. *Thrombosis and Haemostasis* 1998, **80**: 829-835.
4. Neisheim, M., et al. *Thrombosis and Haemostasis* 1997, **78**: 386-391.
5. Hosaka, Y., et al. *Thrombosis and Haemostasis* 1998, **79**: 371-377.


## DEFINITION OF SYMBOLS

| | | | |
|---|---|---|---|
|  | Consult instructions for use | LOT | Batch code / Lot number |
|   | Temperature limitation: Store at 2°C to 8°C | REF | Catalog number |
|  | Manufactured by |  | Use By / Expiration Date |
| CONT | Contains... |   | Contains sufficient for <n> tests |