

ZYMUTEST (TOTAL) TAFI :Ag # RK008A

Thrombin activatable fibrinolysis inhibitor
(Complete ELISA kit for the assay of Total TAFI :Ag)

**FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

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INTENDED USE:

The ZYMUTEST (TOTAL) TAFI :Ag kit is a two-site immuno-assay for measuring human Total TAFI (Thrombin Activatable Fibrinolysis Inhibitor) antigen forms in plasma, or in any fluid where Total TAFI can be present. Zymogen, as well as inactive forms are measured.

This kit is for research use only and should not be used for patient diagnosis or treatment.

ASSAY PRINCIPLE:

ZYMUTEST (TOTAL) TAFI :Ag is a sandwich ELISA specific for human Total TAFI Antigen.

The diluted tested plasma or biological fluid is introduced into a microwell coated with a monoclonal antibody specific for human Total TAFI. When present, this protein is captured onto the solid phase. Following a washing step, the immunoconjugate, which is a goat polyclonal antibody coupled to horse radish peroxidase (HRP), is introduced, and binds to the five epitopes of immobilized Total TAFI. Following a new washing step, the peroxidase substrate, Tetramethylbenzidine (TMB) in presence of hydrogen peroxide (H₂O₂), is introduced and a blue colour develops. The colour turns yellow when the reaction is stopped with Sulfuric Acid. The amount of colour developed is directly proportional to the concentration of human Total TAFI in the tested sample.

TEST SAMPLE:

- Trisodium Citrate or Na₂ EDTA anticoagulated human plasma.
- Any biological fluid where Total TAFI must be measured.

REAGENTS:

1. **COAT:** Micro ELISA plate, containing 12 strips of 8 wells, coated with a mouse monoclonal antibody specific for human Total TAFI, then stabilised; the plate is packed in an aluminium pouch hermetically sealed in presence of a desiccant.
2. **SD:** Two vials containing 50ml of **F-Sample Diluent**, ready to use.
3. **Cal:** Three vials of **Plasma TAFI calibrator**, (normal plasma calibrated with a reference plasma pool), lyophilised.

Each vial, when restored with 0.5 ml distilled water and diluted fifty fold with **F-Sample diluent (SD)**, allows obtaining the calibrator plasma. The exact TAFI concentration is indicated on the flyer provided in the kit.
4. **CI:** One vial containing 0.5 ml of lyophilised **TAFI Control I** (human plasma, high).
5. **CII:** One vial containing 0.5 ml of lyophilised **TAFI Control II** (human plasma, low). The TAFI concentrations and acceptancy ranges for controls are indicated on the flyer provided in the kit.
6. **IC:** Three vials of **Anti (h -Total)-TAFI Ag-HRP immunoconjugate**, a goat polyclonal antibody coupled to HRP, lyophilised.
7. **CD:** One vial of 25 ml of **Conjugate Diluent**, ready to use.
8. **WS:** One vial of 50 ml of 20 fold concentrated **Wash Solution**.
9. **TMB:** One vial of 25 ml peroxidase substrate: 3,3',5,5' - **Tetramethylbenzidine** containing hydrogen peroxide. Ready to use.
10. **SA:** One vial of 6 ml of **0.45M Sulfuric acid (Stop solution)**. Ready to use.

Note: Use only components from kits with the same lot number. Do not mix components from different lots of kits when running the assay.

REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED:

- **8-channel** or repeating **pipette** allowing dispensing 50-300 µl.
- **1-channel pipettes** at variable volumes from 0 to 20 µl, 20 to 200 µl and 200 to 1000 µl.
- **Micro ELISA plate** washing equipment and shaker.
- Micro ELISA plate **reader** with a wavelength set up at 450 nm.
- Distilled water.

REAGENTS PREPARATION, STORAGE AND STABILITY:

In their original packaging box, before use, when stored at 2-8°C, the unopened reagents are stable until the expiration date printed on the box.

1. **Micro ELISA plate:** open the plastic pouch and take off the required amounts of 8 well strips for the test series. When out of the pouch, the strips must be used within 30 minutes. Unused strips can be stored at **2-8°C for 4 weeks** in their original aluminium pouch, in presence of the desiccant, hermetically closed and protected from any moisture, and stored in the provided microplate storage bag (minigrip).
2. **F-Sample Diluent:** It is ready to use. When open, it can be used for **4 weeks**, stored at **2-8 °C**, and provided that any bacterial contamination is avoided during use. This reagent contains 0.05% Kathon CG.
3. **Plasma TAFI calibrator:** restore each vial with **0.5 ml of distilled water**. This undiluted plasma is stable for at least **8 hours** at room temperature and 24H at 2-8°C.
4. **TAFI Control I** (human plasma, high): restore with **0.5 ml** distilled water.
5. **TAFI Control II** (human plasma, low): restore with **0.5 ml** distilled water.

Note: when restored, TAFI controls are stable for **8 hours** at room temperature, **24 hours** at **2-8°C** or **2 months** frozen at **-20°C** or below.

Warning: Plasma TAFI calibrator (3) and controls (4&5) are prepared with normal human plasma. This latter was tested with registered methods and found negative for HIV antibodies, HBs Ag and HVC antibodies. However, no assay may warrant the total absence of infectious agents. Any product of human origin must then be handled with all the required cautions, as being potentially infectious.

6. **Anti (h-Total)-TAFI Ag-HRP immunoconjugate:** each vial must be restored with **7.5 ml of Conjugate Diluent**. Let the pellet completely dissolve before use, and shake the vial gently in order to homogenize the contents. The restored conjugate is stable at least **24 hours** when stored at room temperature or up to **4 weeks** when stored **2-8°C**.
7. **Conjugate Diluent:** It is ready to use. When open, it can be used for **4 weeks**, stored at **2-8 °C**, and provided that any bacterial contamination is avoided during use. This reagent contains 0.05% Kathon CG.
8. **Wash Solution:** Incubate the vial for 15-30 minutes in a water bath, at **37°C**, until complete dissolution of solids, when present. Shake the vial and dilute the amount required 1:20 in distilled water (the 50 ml contained in the vial allow preparing 1 liter of Wash Solution). The Wash Solution must be stored at **2-8°C** in its original vial and used within **4 weeks** following opening. The diluted Wash Solution must be used within **7 days**, when protected from any contamination and stored at 2-8°C. This reagent contains 0.05% Kathon CG.
9. **TMB substrate:** It is ready to use. When open, it can be used for **4 weeks**, stored at **2-8°C**, and provided that any bacterial contamination is avoided during use.
10. **Stop solution:** It is ready to use.

Cautions: Sulfuric acid, although diluted to 0.45M is caustic. As for any similar chemical, handle Sulfuric acid with great care. Avoid any skin and eye contact. Wear protection glasses and gloves when handling.

Note: Bring the kit at room temperature, at least 30 min. before use. Store the unused reagents at 2-8°C.

PROCEDURE:

Specimen collection:

Blood (9 vol.) must be collected on 0.109M citrate anticoagulant (1 vol.); plasma supernatant is decanted following a 20 min. centrifugation at 2,500 g; citrated plasma should be tested within 8 hours or stored frozen at -20°C or below for up to 6 months, and thawed for 15 min. at 37°C just before use. Thawed specimen must be tested within 4 hours. EDTA collected human plasma may also be used. Conditions of storage are the same than those for citrated plasma.

Tested plasma or sample or controls:

The sample must be tested diluted **fifty fold (1:50)** in the F-Sample Diluent. For expected Total TAFI concentrations > 100%, plasma or samples can be tested at a higher dilution, **1:100**, or **1:200**, or **more**. If the dilution factor is D, concentrations obtained must then be multiplied by the complementary dilution factor which is D:50 (i.e., x2 for 1/100, x4 for 1/200 etc...). Controls I and II must be tested diluted **fifty fold (1:50)** as for plasmas.

Calibration:

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

Dilute the plasma calibrator 1:50 with F-SD. Using this 1:50 diluted plasma TAFI Calibrator with a TAFI concentration "C" indicated, for each lot of reagents, on the flyer provided in the kit, prepare the following standard solutions:

Total-TAFI Ag concentration (%)	C	C/2	C/4	C/10	C/20	0
Vol. of 1:50 diluted Plasma TAFI calibrator	1 ml	0.5 ml	0.25 ml	0.1 ml	0.05 ml	0 ml
Vol. of F-Sample Diluent	0 ml	0.5 ml	0.75 ml	0.9 ml	0.95 ml	1 ml

Mix gently for a complete homogenization.

The standard dilutions are stable for at least 4 hours at room temperature.

Assay procedure:

Remove the required number of strips from the aluminium pouch, for the series of measures to be performed. Then put the strips in the frame provided. In the different wells of the micro ELISA plate introduce the reagents and perform the various assay steps as indicated on the following table:

Reagent	Volume	Procedure
Plasma TAFI calibrator or tested sample or controls	200 µl	Introduce the standard solutions or the tested samples in the corresponding micro ELISA plate well.
Incubate for 2 hours at 37°C (a, b)		
Wash Solution (20 fold diluted in distilled water)	300 µl	Proceed to 5 successive washings using the washing instrument.
Conjugate anti (h-Total) –TAFI Ag polyclonal antibody coupled with peroxidase. Restored with 7.5 ml of Conjugate Diluent)	200 µl	Introduce the Anti-(h-Total)-TAFI Ag - HRP immunoconjugate in the micro ELISA plate wells (c).
Incubate for 1 hour at 37°C (a, b)		
Wash Solution (20 fold diluted in distilled water)	300 µl	Proceed to 5 successive washings using the washing instrument.
TMB/H ₂ O ₂ Substrate	200 µl	Immediately after the washing, introduce the substrate into the wells. Note: The substrate distribution, row by row, must be accurate and at exact time intervals (c, d).
Incubate for exactly 5 minutes at room temperature (18-25 °C) (a)		
0.45M Sulphuric Acid	50 µl	Following exactly the same time intervals than for the addition of substrate, stop the colour development by introducing the 0.45M sulphuric acid.
Wait for 10 minutes in order to allow the colour to stabilize and measure absorbance at 450 nm (A450) (e).		

Note:

- In order to allow the complete antigen/antibody reaction, incubating the plates at 37°C is necessary for the Total TAFI Ag ELISA.
- Avoid letting the plate in the bright sunlight during incubations and more particularly during colour development. A micro-ELISA plate shaker can be used.
- Never let the plates empty between the addition of the reagents or following the washing step. The next reagent must be added within 3 minutes, in order to prevent the plate from drying, which could damage the immobilized components. If necessary, keep the plate filled with Wash Solution and empty it just before the introduction of the next reagent. The washing instrument must be adjusted in order to wash the plate gently, and to avoid a too drastic emptying, which could lower plate reactivity.
- For addition of the TMB substrate, the time interval between each row must be accurate and exactly determined. It must be the same when stopping the reaction.
- For bichromatic readings, a reference wavelength at 690 nm or at 620 nm can be used.

RESULTS:

On a linear graph paper plot the **Total TAFI Ag concentrations** (in %) on abscissae and the corresponding absorbance on ordinates.

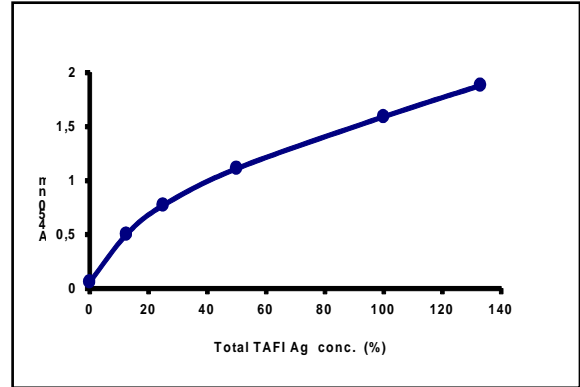
From the curve obtained, deduce directly the Total TAFI Ag concentration in samples tested at the standard 1:50 dilution. When higher dilutions are used (i.e D), the Total TAFI concentration must be multiplied by the complementary dilution factor (i.e., D:50).

Alternatively, an ELISA software (i.e., Dynex, Biolise, etc.) can be used for the calculation of concentrations.

The results obtained should be for research purposes only and not used for patient diagnosis or treatment.

EXAMPLE OF CALIBRATION CURVE:

The calibration curve below is an example only. Users must construct their own calibration curve obtained using their standard dilutions.



BIOCHEMISTRY

TAFI Ag is synthesized in liver. It is a carboxypeptidase which can be activated by thrombin-thrombomodulin complex in an active enzyme, which cleaves the carboxy terminal ends of lysine sites on fibrin. This induces hypofibrinolysis by decreasing the fibrin capacity to bind tPA and plasminogen. TAFI has a molecular weight of 60,000 daltons. Total TAFI Ag concentration in normal human plasma is about 2.5 µg/ml when measured with ZYMUTEST (TOTAL) TAFI :Ag kit.