

# ZYMUTEST proTAFI Ag

# RK037A

(Thrombin Activatable Fibrinolysis Inhibitor (TAFI) Zymogen)  
Complete ELISA kit for the assay of proTAFI in human plasma

For *in vitro* use only

For research use only

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## INTENDED USE:

The ZYMUTEST proTAFI Ag kit is a two-site immuno-assay for measuring human TAFI zymogen (Thrombin Activatable Fibrinolysis Inhibitor), i.e. proTAFI (or proCPU), in plasma, or in any fluid where proTAFI can be present.

## ASSAY PRINCIPLE:

ZYMUTEST proTAFI Ag is a sandwich ELISA specific for human proTAFI.

The diluted tested plasma or biological fluid is introduced into the microwells coated with a monoclonal antibody specific for human proTAFI. When present, this protein is captured onto the solid phase. Following a washing step, the immunoconjugate, which is another monoclonal antibody coupled to horse radish peroxidase (HRP), is introduced, and binds to another epitope of immobilized proTAFI. Following a new washing step, the peroxidase substrate, Tetramethylbenzidine (TMB) in presence of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), 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 proTAFI in the tested sample.

## TEST SAMPLE:

- Trisodium Citrate or Na<sub>2</sub> EDTA anticoagulated human plasma.
- Any biological fluid where proTAFI Ag must be measured.

## REAGENTS:

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

Each vial, when restored with **0.5 ml** distilled water and diluted 1/100 with **F-Sample diluent (SD)**, allows obtaining the calibrator plasma. The exact proTAFI Ag concentration is indicated on the flyer provided in the kit.

4. **CI:** 1 vial containing 0.5 ml of lyophilised **proTAFI Control I** (human plasma, high).
5. **CIII:** 1 vial containing 0.5 ml of lyophilised **proTAFI Control II** (human plasma, low).

**Note:** The proTAFI concentrations and acceptance ranges for controls and calibrator can vary from lot to lot, and are indicated on the flyer provided in the kit.

6. **IC:** 3 vials of **Anti-(H)-proTAFI-HRP immunoconjugate**, a monoclonal antibody coupled to HRP, lyophilised.
7. **CD:** 1 vial of 25 ml of **Conjugate Diluent**, ready to use.
8. **WS:** 1 vial of 50 ml of 20 fold concentrated **Wash Solution**.
9. **TMB:** 1 vial of 25 ml peroxidase substrate: **3,3',5,5' – Tetramethylbenzidine** containing hydrogen peroxide. Ready to use.
10. **SA:** 1 vial of 6 ml of **0.45M Sulfuric acid**. 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.

**Note:** The stability studies for 3 weeks at 30°C show that the reagents can be shipped at room temperature for a short period without damage.

1. **Micro ELISA plate:** open the plastic pouch and take the required amount 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:** 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 proTAFI 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. **proTAFI Control I** (human plasma, high): restore with **0.5 ml** distilled water.
5. **proTAFI Control II** (human plasma, low): restore with **0.5 ml** distilled water.

**Note:** when restored, controls are stable for at least **24 hours** at room temperature, **72 hours** at **2-8°C** or **2 months** frozen at **-20°C** or below.

**Warning:** Plasma controls I and II (4&5) and calibrator (3) 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. **Bovine Serum Albumin (BSA)**, included in some reagents (Cal, CI, CII, IC, CD, SD), was prepared from bovine plasma, which was tested for the absence of infectious agents, and collected from animals free from BSE. However, no assay may warrant the total absence of infectious agents. Any product of biological origin must then be handled with all the required cautions, as being potentially infectious.

6. **Anti-(H)-proTAFI-HRP immunoconjugate:** each vial must be restored with **7.5 ml of Conjugate Diluent**. Let the pellet to be completely dissolved before use, and shake the vial gently in order to homogeneize the content. The restored conjugate is stable for at least **24 hours** at room temperature or for at least **4 weeks** at **2-8°C**.
7. **Conjugate Diluent:** 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:** 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:** 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.

## TESTED SPECIMEN:

**Sample :** Human citrated plasma or biological fluids where proTAFI must be present.

**Collection and preparation:** Blood (9 vol.) must be collected on 0.109M (or 0.129M) citrate anticoagulant (1 vol.); plasma supernatant is decanted following a 20 min. centrifugation at 2,500 g.

**Stability/Storage:** citrated plasma should be tested within **8 hours** or stored frozen at **-20°C** or colder for up to 6 months, and thawed for 15 min. at 37°C just before use.

Note: Refer to GEHT or CLSI recommendations for further instructions on specimen collection, handling and storage. Discard any plasma presenting an unusual aspect (haemolysed, lipaemic aspect...).

EDTA collected human plasma may also be used. Conditions of storage are the same than those for citrated plasma.

### PROCEDURE:

#### Tested plasma or sample or controls:

The sample must be tested diluted a **hundred fold (1:100)** in the F-Sample Diluent (for example 10µl of plasma and 0.99ml of Sample Diluent). For expected proTAFI concentrations > 100 %, plasma or samples can be tested at a higher dilution, **1:200 (D=200), or 1:400 (D=400), or more**. If the dilution factor is **D**, concentrations obtained must then be multiplied by the complementary dilution factor which is **D:100** (i.e. x2 for 1/200, x4 for 1/400 etc...).

Controls I and II must be tested diluted a **hundred fold (1:100)** as for plasmas.

#### Calibration:

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

Reconstitute the calibrator at a defined concentration **C** provided with assay, with **0.5ml** distilled water. Wait until complete homogenization and dilute it **1:100** with F-SD. Using this **1:100** diluted plasma proTAFI Calibrator with a proTAFI Ag concentration "**C**" indicated for each lot of reagents on the flyer provided in the kit, prepare the following standard solutions:

proTAFI Ag concentration (%)	C	C/2	C/4	C/10	0
Vol. of 1:100 diluted Plasma proTAFI calibrator	1 ml	0.5 ml	0.25 ml	0.1 ml	0 ml
Vol. of F-Sample Diluent	0 ml	0.5 ml	0.75 ml	0.9 ml	1 ml

Mix gently for a complete homogenisation.

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

#### Assay procedure:

Remove the required number of 8-well 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 proTAFI calibrator or diluted tested sample or controls or F-SD (blank)	200 µl	Introduce the standard solutions or the tested samples in the corresponding micro ELISA plate well.
<b>Incubate for 2 hours at room temperature (18-25°C) (a)</b>		
Wash Solution (20 fold diluted in distilled water)	300 µl	Proceed to 5 successive washings using the washing instrument. (b)
Conjugate anti proTAFI MoAb coupled with peroxidase. (restored with 7.5 ml of Conjugate Diluent)	200 µl	Introduce the Anti-(h)-proTAFI-HRP immunoconjugate in the micro ELISA plate wells (c).
<b>Incubate for 1 hour at room temperature (18-25°C) (a)</b>		
Wash Solution (20 fold diluted in distilled water)	300 µl	Proceed to 5 successive washings using the washing instrument. (b)
TMB/H <sub>2</sub> O <sub>2</sub> Substrate	200 µl	Immediately after the washing, introduce the substrate into the wells. (b)  <b>Nota:</b> The substrate distribution, row by row, must be accurate and at exact time intervals (a, c).
<b>Incubate for exactly 5 minutes at room temperature (18-25°C) (a)</b>		
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. (c)
Wait for <b>10 minutes</b> in order to allow the colour to stabilize and measure absorbance at <b>450 nm (A450)</b> . Subtract the blank value (d).		

#### Nota:

**Distribute calibrators, controls and tested specimen as rapidly as possible, in order to obtain an homogeneous immunological kinetics for proTAFI binding. A too long delay (>10 min) between the distribution of the first and the last wells may have incidence on immunological kinetics and produce wrong results (underestimated value for the last wells).**

- Avoid letting the plate in the bright sunlight during incubations and more particularly during colour development.. An incubation temperature of 18-25°C must be respected. Results can be affected by a too high (>25°C) or too low (<18°C) temperature, and measured A450 could then be too high or too low. It has to be considered when analyzing the results. A450 values generated in the assay are susceptible to be significantly increased if shaking is used throughout the incubation steps.
- 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 immobilised 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 plates 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 with sulphuric acid.
- For bichromatic readings, a reference wavelength at 690 nm or at 620 nm can be used

#### EXPRESSION OF RESULTS:

On a linear graph paper plot the proTAFI concentrations (%) on abscissa and the corresponding absorbances (A450) on ordinates. Draw the calibration curve (best fit, or second order polynomial regression). Alternatively, a log-log curve can be used (use log-log graph paper).

- Users must construct their own calibration curve, obtained using their calibrator dilutions (See model on the flyer). From the curve obtained, deduce directly the proTAFI concentration for the tested sample when assayed at the standard dilution. For obtaining the proTAFI concentration in a sample tested at a higher or lower dilution, this value must be multiplied by D:100 (e.g.. x2 for a sample tested at the 1:200 dilution , where D=200; or x0.5 for a sample tested at the 1:50 dilution where D=50).

- For controls I and II, tested at the standard 1:100 dilution, concentrations are directly deduced from the calibration curve.

The calibration curve is valid when measured values for the controls are in compliance, within the defined acceptance range indicated on the flyer included in the kit.

- Alternatively, an ELISA software (i.e. Dynex, Biolise, etc...) can be used for the calculation of concentrations (select the best fit curve or a second order polynomial regression curve).

#### EXPECTED RANGE:

- The proTAFI antigen concentration in normal human plasma is usually between 40 and 250% (1).
- Using Zymetest proTAFI Ag, normal plasmas are expected to be between 50 and 200%. There are no variations with gender, or pregnancy. Plasma proTAFI Ag concentrations are stable upon time and do not present nyctemeral variations.

#### BIOCHEMISTRY:

- ProTAFI Ag concentration in normal human plasma is between 4.4 and 15µg/ml (7).
- ProTAFI 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 fibrin (2, 4, 5). This induces hypofibrinolysis by decreasing the fibrin capacity to bind tPA and plasminogen (3). proTAFI has a molecular weight of 60,000 daltons.

#### APPLICATIONS:

- The couple of antibodies used in the Zymetest proTAFI Ag kit is specific for proTAFI (6). This assay does not react with activated forms nor with the activation peptide. It allows to detect an increasing consumption of the proenzyme (9).
- High TAFI Ag concentrations in plasma could induce a hypofibrinolytic status, which can lead to an elevated risk of thrombosis (3).
- Measurement of proTAFI Ag on plasma in order to evaluate a possible hypofibrinolysis resulting from an excess of proTAFI.

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