

# ZYMUTEST (ACTIVATABLE) TAFI

# RK037A

(Thrombin Activatable Fibrinolysis Inhibitor (TAFI) Zymogen)

Complete ELISA kit for the assay of

Activatable TAFI in human plasma

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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## INTENDED USE:

The ZYMUTEST (Activatable) TAFI kit is a two-site immuno-assay for measuring human TAFI (Thrombin Activatable Fibrinolysis Inhibitor) zymogen and fully activatable (or CPU), in plasma, or in any fluid where human TAFI zymogen can be present. Inactive forms of TAFI are measured.

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

## ASSAY PRINCIPLE:

ZYMUTEST (Activatable)TAFI is a sandwich ELISA specific for human TAFI zymogen.

The diluted tested plasma or biological fluid is introduced into the microwells coated with a monoclonal antibody specific for human TAFI zymogen. 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 ActivatableTAFI. 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 TAFI zymogen in the tested sample.

## TEST SAMPLE:

- Trisodium Citrate or Na<sub>2</sub> EDTA anticoagulated human plasma.
- Any biological fluid where (Activatable) 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 TAFI, 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 TAFI 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 TAFI Ag concentration is indicated on the flyer provided in the kit.

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

**Note:** The TAFI 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-Act.)-TAFI-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 (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.

## STORAGE CONDITIONS:

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.

## REAGENTS PREPARATION AND STABILITY:

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 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, 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-Act.)-TAFI-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 for at least 24 hours when stored at room temperature or up to 4 weeks when stored at 2-8°C.
7. **Conjugate Diluent:** Ready to use. When open, it can be used for up to 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.

**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 Activatable TAFI 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 Activatable TAFI 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:

TAFI concentrations are expressed as % of a pool of normal plasmas (which concentration is assigned to 100%). For the TAFI 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 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:

TAFI concentration (%)	C	C/2	C/4	C/10	0
Vol. of 1:100 diluted Plasma TAFI 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 homogenization.

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 TAFI 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>(b)</b>
Conjugate anti (h-Act.)-TAFI 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 <b>(c)</b> .
<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>(b)</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)</b> <b>Note:</b> The substrate distribution, row by row, must be accurate and at exact time intervals <b>(a, c)</b> .
<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. <b>(c)</b>
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 <b>(d)</b> .		

### Note:

**Distribute calibrators, controls and tested specimen as rapidly as possible, in order to obtain homogeneous immunological kinetics for TAFI 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 Activatable TAFI concentration (%) on abscissa and the corresponding absorbance (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 Activatable TAFI concentration for the tested sample when assayed at the standard dilution. For obtaining the Activatable TAFI 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).

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

### BIOCHEMISTRY:

TAFI 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 lysin 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. Activatable TAFI concentration in normal human plasma is variable according to methods used and is usually reported between 2.0 and 15µg/ml.

### APPLICATIONS:

The pair of antibodies used in the Zymutest (Activatable) TAFI kit is specific for human TAFI zymogen, the fully activatable form, whilst all inactive forms are not measured (2). This assay does not react with the activation peptide. It allows detecting the amount of activatable TAFI in the zymogen form.

### REFERENCES:

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Changes compared to the previous version.