# ZYMUTEST™ Total Tissue Factor

**REF RK042A**

**96 tests**

**Enhanced ELISA method for the quantitative determination of Tissue Factor.**

**FOR RESEARCH USE ONLY.**

**DO NOT USE IN DIAGNOSTIC PROCEDURES**

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## INTENDED USE:

The ZYMUTEST™ Total Tissue Factor kit is an enhanced ELISA method for the quantitative determination of Tissue Factor (TF) on plasma and purified milieu, or in any biological fluid where TF can be present.
This kit does not recognize alternatively spliced Tissue Factor (asTF).
**This kit is for research use only and must not be used for patient diagnosis or treatment.**

## SUMMARY AND EXPLANATION:

***Technical:***
Tissue Factor (TF) (also known as Factor III, or thromboplastin), is the physiologic trigger of coagulation. TF binds Factor VIIa to form FVIIa-TF complexes that cleave Factors X and IX, initiating the coagulation cascade. TF is a 47KDa transmembrane protein (SDS-PAGE) constitutively expressed in sub-endothelial cells such as adventitial fibroblasts or smooth muscle cells. TF has three domains: an extracellular domain (aa 1-219), a transmembrane domains (aa 220-242), and a cytoplasmic tail (aa 243-263).

## PRINCIPLE:

In a first step, the TF Assay-Enhancer and the specimen are introduced into a microwell of the plate coated with a highly purified mouse monoclonal antibody specific for human FL-TF. The FL-TF, present in the specimen, binds solid phase by one of its epitopes. In a second step, following a washing step, a monoclonal antibody specific to another epitope coupled to biotin (BIOT Ab), is introduced into the microwells of ELISA plate and binds to immobilized FL-TF on the plate. In a third step, following another washing step, a Horseradish Peroxidase-Streptavidin conjugate (HRP-S) is introduced. Due to its high affinity for biotin, streptavidin binds to the biotinylated antibody. Following a last washing step, the highly sensitive peroxidase substrate (TMB-HS), in presence of hydrogen peroxide (H2O2), is introduced in the microwells and a blue color develops. When the reaction is stopped with Sulfuric Acid, a yellow color is obtained. The color developed is directly proportional to the concentration of human FL-TF in the tested sample.

## REAGENTS:

1. **COAT** **ELISA microplate :** 12x8 contaning 12 strips of 8 wells, coated with a human TF specific monoclonal antibody, stabilized. The microplate is packed in an aluminum pouch in presence of a desiccant.
2. **SD TISSUE FACTOR** **TF Sample Diluent :** 2 vials of 40 mL of diluent, green colored, ready to use. Contains Proclin and BSA.
3. **CAL TISSUE FACTOR** **TF calibrator :** 3 vials of 2 mL of calibrator, lyophilized. Contains BSA.
4. **CI TISSUE FACTOR** **TF Control high :** 1 vial of 1 mL of Tissue Factor Control I, lyophilized. Contains BSA.
5. **CII TISSUE FACTOR** **TF Control low :** 1 vial of 1 mL of Tissue Factor Control II, lyophilized. Contains BSA.
6. **BIOT Ab** **Sheep polyclonal antibody specific of human TF, coupled with biotin, 4 fold concentrated :** 1 vial of 6 mL, lyophilized. Contains BSA.
7. **HRP-S** **HRP-Streptavidin conjugate** : 3 vials of 7.5 mL, lyophilized. Contains BSA.
8. **CD TISSUE FACTOR** **HRP-Streptavidin conjugate diluent :** 1 vial of 25 mL of diluent, ready to use. Contains Proclin.
9. **WS ELISA** **Wash solution :** 1 vial of 50 mL of diluent, 20x 20 fold concentrated. Contains Proclin.
10. **TMB-HS** **3,3',5,5'-Tetramethylbenzidine:** 1 vial of 25 mL of substrate, ready to use. Contains hydrogen peroxide.
11. **Stop** **0.45M Sulfuric acid :** 1 vial of 6 mL, ready to use.

## WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of human and animal origin. Whenever human plasma is required for the preparation of these reagents, approved methods are used to test the plasma for the antibodies to HIV 1, HIV 2 and HCV, and for hepatitis B surface antigen, and results are found to be negative. However, no test method can offer complete assurance that infectious agents are absent. Therefore, users of reagents of these types must exercise extreme care in full compliance with safety precautions in the manipulation of these biological materials as if they were infectious.
- Sulfuric acid, although diluted to 0.45M is caustic. As for any similar chemical, handle Sulfuric acid with great care. Wear protection glasses and gloves when handling. Avoid any skin and eye contact.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.
- Aging studies show that the reagents can be shipped at room temperature without degradation.
- This device of *in vitro* use is intended for professional use in the laboratory.

## REAGENT PREPARATION:

Allow the strips and reagents to stabilize for at least 30 min at room temperature before use. Gently remove the freeze-drying stopper, to avoid any product loss when opening the vial.

**COAT** Open the aluminum pouch and take off the required amounts of strips for the test series. Open the seals of the strips required for the test series. The strips must be used within 30 minutes

Reconstitute the contents of each vial with exactly:
**CI TISSUE FACTOR** ➔ **1 mL of distilled water** to obtain a solution containing about 350 pg/mL of recombinant human TF (see the flyer provided in the kit). Shake vigorously until complete dissolution
**CII TISSUE FACTOR** ➔ **1 mL of distilled water** to obtain a solution containing about 75 pg/mL of recombinant human TF (see the flyer provided in the kit). Shake vigorously until complete dissolution.
**CAL TISSUE FACTOR** ➔ **2 mL of SD TISSUE FACTOR in order to obtain a solution containing** "C" pg/mL **of recombinant human TF** (**about 600 pg/mL, see the flyer provided in the kit**). Shake vigorously until complete dissolution.
**BIOT Ab** ➔ **6 mL of SD TISSUE FACTOR at least 15 minutes before use. Shake vigorously until complete dissolution,** then withdraw the quantity of biotinylated antibody necessary for the test and dilute it 4 fold in **SD TISSUE FACTOR**. **For example, for 4 strips, withdraw 1.8 mL of biotinylated antibody and introduce 5.4 mL of SD TISSUE FACTOR.**
**HRP-S** ➔ **7,5 mL of CD TISSUE FACTOR at least 15 minutes before use.** Shake gently until complete dissolution.

**SD TISSUE FACTOR** **TMB-HS** **Stop** **CD TISSUE FACTOR**
Reagent ready to use.

**WS ELISA** Shake the vial and dilute the wash solution **1:20 in distilled water** (the 50 mL of concentrated solution allow to prepare 1 Litter of wash solution after dilution). Incubate, if necessary, the vial in a water bath at **37°C**, until complete dissolution of solids.

## STORAGE AND STABILITY:

Unopened reagents should be stored at 2-8°C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

**COAT** Unused strips can be stored at 2-8°C **for 4 weeks** in their original aluminum pouch (hermetically closed, in presence of the desiccant), stored in the provided plastic microplate storage bag (minigrip), protected from any moisture.

Reagent stability after reconstitution, free from any contamination or evaporation, and stored closed, is of:
**CAL TISSUE FACTOR** **CI TISSUE FACTOR** **CII TISSUE FACTOR**
➔ **72 hours at** 2-8°C.
**8 hours** at room temperature (18-25°C).
**2 months** frozen at -20°C or less*

**BIOT Ab** ➔ **4 weeks** at 2-8°C.
**24 hours** at room temperature (18-25°C).
**6 months** frozen at -20°C or less*

**HRP-S** ➔ **2 weeks** at 2-8°C.
**24 hours** at room temperature (18-25°C).
**2 months** frozen at -20°C or less*

*Thaw only once, as rapidly as possible at 37°C and use immediately.

Reagent stability after opening, free from any contamination or evaporation, and stored closed, is of:
**SD TISSUE FACTOR** **CD TISSUE FACTOR** **TMB-HS**
➔ **4 weeks** at 2-8°C.

**WS ELISA** ➔ **4 weeks** at 2-8°C.
**7 days** at 2-8°C for the diluted solution.

**Stop** ➔ **8 weeks** at 2-8°C.

## REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

***Reagents:***
- Distilled water.

***Materials:***
- 8-channel or repeating pipette allowing dispensing volumes of 50-300 µL.
- 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.

## SPECIMEN COLLECTION AND PREPARATION:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture. Discard the first tube.
Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI GP44-A4[4] (and CLSI H21-A5[5]) guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references[4,5].

## PROCEDURE:

**Assay method:**

1. Specimens and controls should be tested undiluted. For specimens other than human plasma, the dilution must be adjusted to have a final TF concentration between 25 and 500 or "C" pg/mL. Dilutions must be done in **SD TISSUE FACTOR**.

2. Using the Calibrator **CAL TISSUE FACTOR** concentration "C" in pg/mL, prepare the calibration range as described in the table below:

| TF concentration (pg/mL) | C | C:2 | C:4 | C:10 | C:20 | 0 |
|---|---|---|---|---|---|---|
| Vol. of TF calibrator | 1 mL | 0.5 mL | 0.25 mL | 0.1 mL | 0.05 mL | 0 mL |
| Vol. of Sample diluent | 0 mL | 0.5 mL | 0.75 mL | 0.9 mL | 0.95 mL | 1 mL |

Mix for homogenization.
The standard dilutions are stable for **4 hours** at room temperature (18-25°C).

3. Put strips in the frame provided. Introduce the reagents in wells and perform the assay as indicated on the following table:

| Reagent | Volume | Procedure |
|---|---|---|
| **SD TISSUE FACTOR** | **100 µL** | Introduce the **SD TISSUE FACTOR** in the micro ELISA plate wells |
| **Specimens** or **CAL TISSUE FACTOR** or **CI TISSUE FACTOR** **CII TISSUE FACTOR** or **SD TISSUE FACTOR** **(blank)** | **100 µL** | Introduce the standard solutions or the tested specimen in the corresponding micro ELISA plate well |
| **Incubate for 2 hours at 37°C (a) (or overnight at room temperature (18-25°C)) (a)** | | |
| **WS ELISA** | **300 µL** | Proceed to 5 successive washings **(b)** |
| **BIOT Ab** | **200 µL** | Introduce the Biotinylated Antibody diluted 1:4 in **SD TISSUE FACTOR** in the micro ELISA plate wells |
| **Incubate for 2 hours at 37°C (a)** | | |
| **WS ELISA** | **300 µL** | Proceed to 5 successive washings **(b)** |
| **HRP-S** | **200 µL** | Introduce the **HRP-S** in the micro ELISA plate wells |
| **Incubate for exactly 30 minutes at room temperature (18-25°C) (a)** | | |
| **WS ELISA** | **300 µL** | Proceed to 5 successive washings **(b)** |
| **TMB-HS** | **200 µL** | Immediately after the washing, introduce the substrate into the wells. **(b)** ***Note:*** The substrate distribution, raw by raw, must be accurate and at exact time intervals **(c)**. |
| **Incubate for exactly 15 minutes at room temperature (18-25 °C) (a)** | | |
| **Stop** | **50 µL** | Following exactly the same time intervals, raw to raw, than for the addition of substrate, stop the reaction by introducing the 0.45M sulfuric acid **(c).** |
| **Wait for 10 minutes in order to allow the colour to stabilize then measure absorbance at 450 nm. Subtract the blank values (d).** | | |

**Distribute calibrator dilutions, controls and specimens as rapidly as possible, in order to obtain homogeneous kinetics of the dosage. A too long delay (>10 min) between the first and the last distribution wells may have incidence on immunological kinetics and produce inaccurate results (underestimated value for the last wells).**

**(a)** Avoid letting the plate in the bright sunlight during incubations and more particularly during colour development. A micro ELISA plate shaker can be used.

**(b)** 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 and reduce the reactivity plate. 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 damage coating and lower plate reactivity.

**(c)** For addition of the substrate, the time interval between each row must be accurate and exactly determined.

**(d)** For bichromatic readings, a reference wavelength at 690 nm or at 620 nm can be used.

In case of the full plate is used, distribute the calibrator's dilutions on the center of the plate to reduce the kinetics effect.

## QUALITY CONTROL:

Using quality controls allows validating the method compliance, as well as the homogeneous of assays for a same lot of reagents.
Quality control plasmas must be included in each series, as per good laboratory practice, in order to validate test results. A new calibration curve must be carried out for each test series.
Each laboratory can establish acceptance ranges and verify expected performances in its analytical system.

## TRACEABILITY:

The concentration of TF, for each new lot of calibrators and controls, is established against the internal reference which concentration has been precisely determined. The calibrator and controls are prepared with recombinant full-length human Tissue Factor (1-263).

## RESULTS:

- Obtained OD450 can vary according to the effective temperature during the assay run.
- Plot the calibration curve with the OD 450 nm along the Y-axis and the TF concentration in pg/mL, along the X-axis by choosing the "best fit" interpolation mode (refer to the flyer in the kit).
- Results are expressed with the obtained OD450 for specimens and controls using the calibration curve.
- The concentration of TF (pg/mL) in the test specimen (non diluted) is directly inferred from the calibration curve.
- If other dilutions are used, the level obtained should be multiplied by the additional dilution factor used.
- Alternatively, a specific software (i.e., Dynex, Biolise, etc...) can be used for the calculation of concentrations.

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

## LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully.
- Any reagent presenting an unusual appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- If the washing step is not correctly performed, this might induce elevated background and a high absorbance value of the negative control. In order to avoid non-specific color development, check that the washing step is performed efficiently.

## PERFORMANCES:

- Dynamic range: about 3 pg/mL at 700 pg/mL.
- The detection threshold is ≤ 10 pg/mL.
- CV intra essais : (N= 12): CV = 9% pour **CI TISSUE FACTOR**
  CV = 8% pour **CII TISSUE FACTOR**
- CV inter essais : (N= 10) : CV = 6% pour **CI TISSUE FACTOR**
  CV = 7% pour **CII TISSUE FACTOR**
- Recovery of full length TF in plasma: about 80% for undiluted plasmas.
- Recovery of truncated TF (1-219) in plasma: about 80% for undiluted plasmas.
- Specificity: the reactivity is inhibited in plasmas following the addition of a monoclonal antibody specific for human TF.
- Interferences: the kit has been optimized to minimize the interference of the potential presence of heterophilic antibodies in plasmas, which could otherwise result in an abnormal overestimation of TF concentration.
- Reactivity: the kit recognizes full length TF (1-263) and the extracellular domain of TF (1-219). It does not recognize asTF.

## REFERENCES:


1. Parhami-seren B. *et al*. Immunologic quantitation of tissue factors. J. Thromb. Haemost. 2006.
2. Mackman N. The many faces of Tissue Factor. J. Thromb. Haemost. 2009.
3. Edgington TS. *et al.* The structural biology of expression and function of tissue factor. Thromb Haemost. 1991.
4. CLSI Document GP44-A4: "Procedures for the handling and processing of blood specimens for common laboratory tests".
5. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008.


## SYMBOLS:

Symbols used and signs listed in the ISO 15223-1 standard, see Symbol definitions document.

**CD TISSUE FACTOR** | **SD TISSUE FACTOR** | **WS ELISA**

H317 : May cause an allergic skin reaction.