ZYMUPHEN MP-TF

Ref 521196
(96 tests)

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Functional assay for the measurement of Tissue Factor-bearing microparticles procoagulant activity

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

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INTENDED USE:

The ZYMUPHEN MP-TF kit is a bio-immunoassay for the *in vitro* determination of the procoagulant activity of microparticles exposing Tissue Factor (MP-TF), in human plasma and in purified milieu.
This kit is for research use only and should not be used for patient diagnosis or treatment.

SUMMARY AND EXPLANATION:

Cellular microparticles are fragments of the plasma membrane released by virtually all cells when subject to a number of stress conditions, including cellular activation and apoptosis.
Tissue Factor (TF, also known as coagulation Factor III, or thromboplastin) is the physiologic trigger of blood coagulation. TF binds to Factor VIIa to form FVIIa-TF complexes that cleave Factors IX and X, initiating the whole coagulation cascade[1]. This transmembrane protein of 47KDa (SDS-PAGE) is constitutively expressed in sub-endothelial cells such as 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). It exists also as an alternatively spliced soluble form (1-206).

PRINCIPLE:

In a first step, the **AE-MP-TF** solution and the sample to test are introduced into the wells of the microplate coated with a murine monoclonal antibody, specific for human Tissue Factor (TF, extracellular domain), and which does not interfere with TF activity. MP-TF present in the sample bind to the solid phase through an epitope localized in the extracellular domain of TF. Following an overnight incubation and an automated washing step, wash solution is immediately introduced into the wells. Then, Factor VIIa (**R1**) and Factor X (**R2**) are the introduced in the microplate wells. The TF-FVIIa complex forms and subsequently activates Factor X into activated Factor X (FXa), on the surface of the anionic phospholipids present in the microparticle, and in presence of calcium ions. The MP-TF concentration is the limiting factor. In a third step, a specific substrate for Factor Xa (CS11-65) (**R3**) is introduced and reacts with Factor Xa to produce a yellow color. The absorbance, recorded at 405nm on a spectrophotometer, is directly proportional to the amount of MP-TF present in the sample to test.

REAGENTS:

- **COAT: Micro ELISA plate:** containing **12 strips of 8 wells**, coated with a monoclonal antibody specific for human TF, then stabilised. The microplate is packed in an aluminium pouch hermetically sealed in presence of a desiccant.
- **SD-MP-TF: MP-TF Sample Diluent:** 1 vial containing **50 mL** of the sample diluent, blue colored, ready to use. Contains BSA.
- **AE-MP-TF: MP-TF Assay Enhancer:** 1 vial containing **25 mL** of MP-TF Assay Enhancer, colorless**,** ready to use. Contains BSA.
- **CAL: MP-TF Calibrator:** 2 vials of **2 mL** of calibrator, containing "C" pg/mL of MP-TF (about 25 pg/mL equivalent TF, see flyer), lyophilized. Contains BSA.
- **CI/CII: Control High (CI) and Low (CII):** 1 vial of each control, containing **2 mL** of control high and low, lyophilized. Contains BSA.
- **R1: Factor VIIa :** 2 vials of recombinant human Factor VIIa, lyophilised. Contains BSA.
- **R2: Factor X:** 2 vials of highly purified human Factor X, lyophilised. Contains BSA.
- **R3: Factor Xa specific chromogenic substrate (CS 11(65)):** 2 vials of specific substrate, lyophilised.
- **WS-MP-TF: Wash Solution:** 1 vial of **50 mL** of diluent (Wash Solution MP-TF), 20 fold concentrated.
- **CA: Citric Acid 2%:** 1 vial of **6 ml** of diluent (Stop solution), ready to use.

The MP-TF concentration for each controls (CI/CII) and the calibrator (CAL) is indicated on the flyer provided in the kit. The microparticles concentrations for the controls and the calibrator may vary from lot to lot. For the assay, refer to the concentration indicated on the flyer provided in the kit used.

SD-MP-TF, AE-MP-TF and WS-MP-TF reagents contain low concentration of Sodium azide (0.9 g/L), see CAUTIONS AND WARNINGS.

WARNINGS AND PRECAUTIONS:

- Any product of biological origin must then be handled carefully with all the required cautions, as being potentially infectious.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.
- If the substrate becomes yellow, this indicates the presence of a contaminant. It must be rejected, and a new vial must be used.
- The disposal of waste materials must be carried out according to current local regulations
- Use only reagents from kits with the same lot number. Do not mix reagents from kits with different lots when running the assay; they are optimized for each lot of kits.
- Handle the reagents with care to avoid contamination during use. If possible, avoid reagent evaporation during use by limiting the liquid-air exchange surface.
- In order to preserve the stability of the reagents, close the vials with their original screw cap following each use.
- Stability studies for 3 weeks at 30°C show that the reagents can be shipped at room temperature for a short period without damage.
- The bovine plasma used to prepare the BSA has been tested by recorded methods and is certified free of infectious agents, in particular the causative agent of bovine spongiform encephalitis.
- For *in vitro* use.

SD-MP-TF, AE-MP-TF: H317: May cause an allergic skin reaction.

PREPARATION AND STABILITY OF REAGENTS:

Bring the kit at room temperature, at least 30 min before the assay. Store the unused reagents at 2-8°C. Remove carefully the stopper for lyophilized products, in order to avoid any loss of powder when opening the vials.

- **COAT :** Open the aluminium 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 plastic microplate storage bag (minigrip).

- **SD-MP-TF**, **AE-MP-TF:** Ready to use. These reagents contain 0.05% Kathon CG and 0,9 g/L of sodium azide.
  Stability of reagents after opening, provided that any contamination or evaporation is avoided, kept in its original vial is of:
  - **4 weeks** at 2-8°C.

- **CAL :** Reconstitute each vial with exactly **2 mL of SD-MP-TF** in order to obtain a solution containing "C" pg/mL of human TF (about 25 pg/mL of TF, see the flyer provided in the kit for the exact concentration).
  Homogenize the reagent before each use.
  Stability of reagent after reconstitution, provided that any contamination or evaporation is avoided, kept in its original vial is of:
  - **24 hours** at 2-8°C.
  - **8 hours** at room temperature (18-25°C).
  - **2 months** frozen at -20°C or below*

- **CI/CII :** Reconstitute each vial with exactly **2 mL of SD-MP-TF** in order to obtain a solution containing about 16 pg/mL of MP-TF in control high and about 5 pg/mL in control low (see the flyer provided in the kit for the exact concentration).
  Stability of reagents after reconstitution, provided that any contamination or evaporation is avoided, kept in its original via is of:
  - **24 hours** at 2-8°C.
  - **8 hours** at room temperature (18-25°C).
  - **2 months** frozen at -20°C or below*

- **R1, R2 :** Reconstitute each vial with exactly **1.5 mL of distilled water**, shake thoroughly for complete dissolution.
  Let the reagent stabilize for 30 min at room temperature (18-25°C) while shaking from time to time.
  Homogenize reagents before each use.
  Stability of reagents after reconstitution, provided that any contamination or evaporation is avoided, kept in its original vial is of:
  - **24 hours** at 2-8°C.
  - **8 hours** at room temperature (18-25°C).
  - **2 months** frozen at -20°C or below*

- **R3 :** Reconstitute each vial with exactly **3 mL of distilled water**, shake thoroughly for complete dissolution.
  Let the reagent stabilize for 30 min at room temperature (18-25°C) while shaking the vial from time to time.
  Homogenize the reagent before each use.
  Stability of reagent after reconstitution, provided that any contamination or evaporation is avoided, kept in its original vial is of:
  - **24 hours** at 2-8°C.
  - **8 hours** at room temperature (18-25°C).
  - **2 months** frozen at -20°C or below*

- **WS-MP-TF :** Incubate the vial in a water bath at 37°C until complete dissolution of solids. Shake the vial and dilute the Wash Solution at 1:20 in distilled water (the 50 mL contained in the vial allow preparing 1000 mL of Wash Solution after dilution).
  Stability of wash solution, provided that any contamination or evaporation is avoided, kept in its original vial is:
  - **4 weeks** at 2-8°C.

  Stability of diluted wash solution, provided that any contamination or evaporation is avoided, kept in its original vial is:
  - Once opened, **7 days** at 2-8°C.

  This reagent contains 0.9 g/L of sodium azide.

- **CA :** Ready to use.

*Thaw once as rapidly as possible at 37°C, adapt incubation duration to the volume of reagent. The stability of the thawed reagent should be verified in the working conditions of the user laboratory.

STORAGE CONDITIONS:

Unopened reagents must be stored at 2-8°C, in their original packaging box. They are then usable until the expiration date printed on the kit.

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.

Materials:

- **Micro ELISA plate washing equipment (and agitator) able to distribute 100µL of wash solution at the end of the washing (critical step)**.
- Spectrophotometer or automatic instrument for chromogenic assays with a wavelength set up at 405 nm (reading range up to 4u of OD).
- Water-bath, incubator at 37°C.
- Stopwatch; Calibrated pipettes.

SPECIMEN COLLECTION AND PREPARATION:

Preparation and storage of specimens must be performed according to the current local regulations.

- Specimens:

Human plasma obtained from trisodium citrate anticoagulated blood.

- Collection:

Blood (9 volume) must be collected on trisodium citrate anticoagulant (1 volume) (0.109M), with caution, through a net venipuncture. The first tube must be discarded.

- Centrifugation **(Samples must never be stored or centrifuged at 4°C):**

Within 2 hours, use a validated method in the laboratory to obtain a plasma with no platelet, for exemple a minimum of 15 minutes at 1500g at room temperature (18-25°C), then recovered plasma supernatant is rapidly centrifuged for 2 minutes at 13000g at room temperature (18-25°C). Plasma is obtained by collecting the supernatant, avoiding any contact with the platelet pellet.

- Storage of plasma:
  - 4 hours at room temperature (18-25°C).
  - 1 month at -20°C.
  - 18 months at -70°C.

Frozen plasma specimens should be rapidly thawed at 37°C, then gently mixed and tested immediately. Resuspend any precipitation by thorough mixing immediately after thawing and before testing.

PROCEDURE:

Assay method:

1. Reconstitute the calibrator and controls using the specific package inserts. Calibrators should be diluted using SD-MP-TF buffer as described in the table below in order to establish the calibration range ("C" = defined MP-TF concentration):

Calibrator	C	C:2	C:4	C:10	C:20	0
Volume calibrator	1 mL	0.5 mL	0.25 mL	0.1 mL	0.05 mL	0 mL
Volume SD-MP-TF	0 mL	0.5 mL	0.75 mL	0.9 mL	0.95 mL	1 mL

Delicately mix to obtain a homogenous solution.
The calibration dilutions should be extemporaneously prepared to obtain optimal performances.

2. The **plasma samples and controls** must be tested **undiluted**. For other types of sample, the dilution factor must be adjusted to have a final concentration between 0 and "C" pg/mL (about 25 pg/mL of Tissue Factor). Dilution must be done in **SD-MP-TF.**
Run the calibration curve and test it with quality controls. The exact concentration of the calibrators and controls is indicated for each lot on the flyer provided with the kit.

3. Take off the required amount of strips on the aluminum pouch and place them in the framework provided. Incubating the reagents R1, R2 and R3 at least 15 minutes at 37°C before use. Introduce reagents into the wells of microbarrettes and perform the assay as described in the table below :

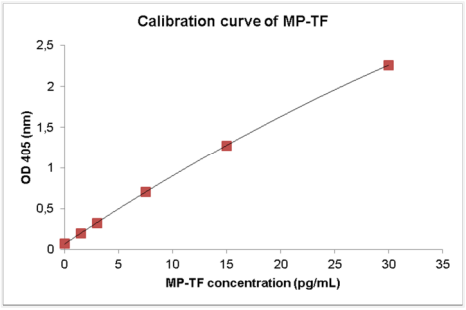
Reagents	Volume	Procedure
MP-TF-Assay Enhancer (AE-MP-TF)	**200 µL**	Introduce the Assay Enhancer
MP-TF calibrator, or undiluted plasma sample and controls, or SD-MP-TF (blank)	**20 µL**	Introduce standards, controls or samples
Incubate overnight at room temperature (18-25°C) kept under adhesive seal (a)		
Wash solution (WS MP-TF) (diluted 20 times in distilled water before use)	**300 µL** per well	Proceed to 5 successive washings using an **automate**,
	100 µL	and arrange the automate to distribute **immediately, in every well, 100µL of wash solution (critical step !!) (b)**
R1 (beforehand reconstitute in 1,5 mL of distilled water and pre-incubated at 37°C)	**25 µL**	**Do not discard the wash solution**, introduce Factor VIIa **(c)**
R2 (beforehand reconstitute in 1,5 mL of distilled water and pre-incubated at 37°C)	**25 µL**	Introduce Factor X **(c)**
Incubate precisely for 2 hours at 37°C		
R3 (beforehand reconstitute in 3 mL of distilled water and pre-incubated at 37°C)	**50 µL**	Introduce the substrate, keeping the same time interval between each strip **(d).**
Let the substrate to react for exactly **2 hours exactly at 37°C**		
Citric acid 2% (CA)	**50 µL**	Following exactly the same time intervals than for the addition of substrate, stop the colour development by introducing 2% Citric Acid **(d).**
Wait for 10 minutes in order to allow the colour to stabilize and measure absorbance at 405 nm (A405). Subtract the blank value (e, f).		

Note:

a. Adhesive seal permits to avoid evaporation of the sample during the overnight incubation.
b. **Automated washing is required. Never let the well of ELISA plate empty following the washing step to prevent the MP-TF oxidation**, which lead an important and heterogeneous loss of reactivity or aspecific reactions.**The washing instrument must be adjusted to fill each microwell with 100 µL of wash solution, immediately after washing.** Adjusting the washing instrument to perform a soft washing. Too violent purge wells during aspiration can damage the coating and reduce the reactivity.
c. To facilitate pipetting, it is possible to pool R1 and R2 vials just before (within 5 minutes) adding them into the microplate, and to distribute 50 µl of the mixture instead of 25 µL of each reagent.
d. During substrate distribution, the same time interval between each strip must be carefully respected. The same time interval must then be respected for the reaction arrest by the citric acid.
e. **The assay can be used with a kinetics mode (for example ΔA405 measured between 20 seconds and 2 hours) on a microplate reader allowing plate incubation at 37°C.**
f. For bichromatic readings, a reference wavelength at 620 nm or at 690 nm can be used.

CALIBRATION:

The calibration curve below is indicated as an example only. The calibration curve generated for the series of measures performed must be used.



QUALITY CONTROL:

Using quality controls, allows validating the calibration curve, as well as the homogeneous reactivity from run to run, when using a same lot of reagents.
Include quality controls 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 and when quality controls values are measured outside the acceptance range determined for the method.
Each laboratory should establish acceptance range and verify expected performances in its analytical system.

RESULTS:

- For the manual method, draw the calibration curve on a linear graph paper plot, with on abscissae, the MP-TF concentration (**pg/mL**) and on ordinates OD at 405 nm.
- For plasma samples tested undiluted, deduce directly the MP-TF concentrations from the calibration curve obtained. If another dilution factor has been used, the value read on the calibration curve must be multiplied by the dilution factor (i.e.: x4 if the samples are tested at the 1:4 dilution).
- The interpretation mode used is indicated in the CoA of the kit.
- Alternatively, specific software (i.e., Dynex, Biolise, etc...) can be used for the calculation of concentrations.
- Results are expressed in **pg/mL.**

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

LIMITATIONS:

- In order to get the optimal assay performances and adhere to specifications, the procedural instructions validated by HYPHEN BioMed must be strictly respected. It is responsibility of the user laboratory to validate any modification to those instructions for use.
- Any reagent presenting an unusual aspect or contamination signs must be rejected.
- Any suspect sample or presenting activation signs must be rejected.
- Any plasma containing a coagulum or contamination signs must be rejected.
- If the washing step is not correctly performed, the negative control can produce a high absorbance value. In order to avoid non-specific colour development, check that the washing step is performed efficiently.

PERFORMANCES:

The lower limit of detection is ≤ 1 pg/mL.
Specificity: MP-TF reactivity is inhibited by an anti-TF polyclonal antibody spiked into the sample. The kit does not react with truncated TF (1-219), nor synthetic liposomes.
Interferences: The kit has been optimized to avoid interferences due to the microparticles that do not bear Tissue Factor at the surface.

REFERENCES:

1. Wang *et al*., "Levels of microparticles tissue factor activity correlate with coagulation activation in endotoxemic mice", J Thromb Haemost. 2009.

SYMBOLS:

Used symbols and signs listed in the ISO standard 15223-1, refer to the Definition of Symbols document.

Changes compared to the previous version.