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ZYMUTEST PAI-1 Antigen

 # RK012A-RUO

(Complete ELISA kit for Tissue- Plasminogen Activator Inhibitor Type I)

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

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INTENDED USE:

The ZYMUTEST PAI-1 Antigen kit is a one step, two site immuno-assay for measuring human Tissue- Plasminogen Activator Inhibitor, type I (PAI-1) in plasma, or in any fluid where PAI-1: Ag can be present. **This assay is for research use only and should not be used for patient diagnosis or treatment.**

ASSAY PRINCIPLE:

First, the immunoconjugate, which is a monoclonal antibody specific for PAI-1: Ag coupled to horse radish peroxidase (HRP), is introduced into the microwells coated with another monoclonal antibody specific for PAI-1: Ag. Then, the diluted tested sample is immediately introduced, and the immunological reaction starts. When present, PAI-1: Ag binds onto the monoclonal antibody coated solid phase through one epitope, and fixes the second monoclonal antibody coupled to HRP by another epitope. Following a washing step, the peroxidase substrate, 3,3',5,5' – Tetramethylbenzidine (TMB), in presence of hydrogen peroxide (H_2O_2), is introduced and a blue colour develops. When the reaction is stopped with Sulfuric Acid, a yellow colour is obtained. The amount of colour developed is directly proportional to the concentration of human PAI-1: Ag in the tested sample.

TEST SAMPLE:

- Trisodium Citrate or Na_2 EDTA anticoagulated human plasma.
- Any biological fluid where PAI-1: Ag must be measured.

REAGENTS:

1. **COAT: Micro ELISA plate,** containing 12 strips of 8 wells, coated with a murine monoclonal antibody specific for human PAI-1: Ag, 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. **STD:** 3 vials of **PAI-1 Standard**, lyophilised. When restored with 2 ml of F-Sample Diluent, a solution containing about **10 ng/ml** of recombinant human PAI-1: Ag is obtained. The exact PAI-IAg concentration is indicated on the flyer provided in the kit.
4. **CI:** 1 vial containing **1 ml** of lyophilised **Plasma PAI-1 Control I High** (human plasma).
5. **CII**: 1 vial containing **1 ml** of lyophilised **Plasma PAI-1 Control II Low** (human plasma).

Note *:* The PAI-1: Ag concentrations and acceptancy ranges for controls can vary from lot to lot, and are indicated on the flyer provided in the kit.

6. **IC:** 3 vials of **Anti-(h)-PAI-1-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 a same kit lot. Do not mix components from different lots, 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. **PAI-1 Standard:** restore each vial with **2 ml** of F-Sample Diluent in order to obtain a solution containing about **10 ng/ml.** This solution is stable for at least **8 hours** at room temperature.
4. **Plasma PAI-1 Control I** (human plasma, high): restore with **1 ml** distilled water.
5. **Plasma PAI-1 Control II** (human plasma, low): restore with **1 ml** distilled water.

Note: when restored, PAI-1 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 controls I and II (4&5) and standard (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. Any product of human origin must then be handled with all the required cautions, as being potentially infectious.

6. **Anti-(h)-PAI-1-HRP immunoconjugate**: each vial must be restored with **4 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**: 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.
The stability studies at 30°C show that the reagents can be shipped at room temperature without damage.

PROCEDURE:

Specimen collection:

Blood (9 vol.) must be collected, through a clean venipuncture avoiding any blood activation, on 0.109M citrate anticoagulant (1 vol.) or on CTAD (Citrate-Theophylline-Adenoside-Dipyridamole) tubes; plasma supernatant is decanted following a 20 min. centrifugation at 2,500 g; in order to avoid platelet activation, it is recommended to collect 1:3 of plasma supernatant by aiming the pipette tip at the middle of this supernatant; 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.
PAI-1: Ag can be released from platelets upon activation or disruption. In order to avoid an overestimation of PAI-1, platelets must be accurately removed. Use of CTAD tubes, which prevent from platelet activation, is recommended.
In order to avoid diurnal variations, PAI-1 should be tested on fasting samples, collected at morning.
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 **five fold (1:5)** in the F-Sample Diluent. For expected PA-1: Ag concentrations > 50 ng/ml, plasma or samples can be tested at a higher dilution, **1:10, or 1:20, or more**.

Plasma Controls I and II must be tested diluted **five fold (1:5)**, with F-Sample Diluent.

Calibration:

Using the **"C" ng/ml PAI-1** standard provided in the kit, prepare the following standard solutions.

PAI-1 concentration (ng/ml)	C	C/2	C/4	C/10	C/20	0
Vol. of PAI-1 Standard	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 homogenisation.

The standard dilutions are stable for at least **6 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
Conjugate anti (h)-PAI-1-HRP. (Restored with 4 ml of conjugate Diluent)	**100 µl**	Introduce the Anti-(h)-PAI-1- HRP immunoconjugate in the micro ELISA plate wells
PAI-1 Standard or tested sample or F- sample diluent (blank)	**100 µl**	Introduce **immediately** the standard solutions or the tested samples in the corresponding micro ELISA plate well
Mix gently on a plate shaker or manually and incubate for 1 hour at room temperature		
Wash Solution (20 fold diluted in distilled water)	**300 µl**	Proceed to 5 successive washings using the washing instrument. **(a)**
TMB/H_2O_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 **(b, c)**.
Incubate for exactly 5 minutes at room temperature (18-25 °C) (c)		
0.45 M Sulfuric Acid (5)	**50 µl**	Following exactly the same time intervals than for the addition of substrate, stop the colour development by introducing the 0.45M sulfuric acid **(b).**
Wait for **10 minutes** in order to allow the colour to stabilize and measure absorbance at **450 nm (A450)**. Subtract the blank value **(d)**.		

Note:

a. 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.

b. 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.

c. Avoid letting the plate in the bright sunlight during incubations and more particularly during colour development. A micro-ELISA plate shaker can be used.

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

TWO STEP METHOD:

- The assay can also be performed with a two step method. The calibration curve is from **0 to C ng/ml** (as for the one step method), the PAI-1 standard being reconstituted with **2 ml** of F-Sample Diluent (**SD**).
- The immunoconjugate (**IC**) must be reconstituted with **7.5 ml** of Conjugate Diluent (**CD**).
- Tested plasma must be assayed at a five fold (**1:5**) dilution or at higher dilutions in F-Sample Diluent (**SD**), if required.
- In each microwell, **100 µL** of SD are introduced, immediately followed by **100µL** of the calibration solution (prepared as for the one step method) or 100µL of the diluted tested plasma. Following a **1 hour** incubation at room temperature (18-25°C) and a washing step, **200µl**/well of immunoconjugate (**IC**) are introduced. Following a new **1 hour** incubation at room temperature and a washing step, the colour development with TMB (**200µl**/well) is allowed to develop for **5 min**, and is then stopped with **50 µl** of 0.45M sulfuric acid (**SA**). A450 is then measured. Washing and operating cautions, as well as results interpretation, are the same as recommended for the one step method.

EXPRESSION OF RESULTS:

- On a linear graph paper plot the **PAI-1: Ag concentrations (ng/ml)** on abscissa and the corresponding absorbances (**A450**) on ordinates.
- Users must construct their own calibration curve, obtained using their standard dilutions. From the curve obtained, deduce the PAI-1: Ag concentration for the tested sample. For obtaining the PAI-1: Ag concentration in this sample, the value read on the calibration curve must be multiplied by the dilution factor (**i.e. 5,10, 20.....**) (See model on the flyer).
- For controls I and II, the concentrations measured must be multiplied by **5**.
- 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.**

BIOCHEMISTRY:

- PAI-1 is a single chain glycoprotein, synthesised by endothelial cells and hepatocytes and with a molecular weight of 50,000 daltons. In plasma it is stabilised by binding to vitronectin, or circulates as inactive complexes with tPA or uPA. PAI-1 is also present in platelets, but in the latent form. PAI-1 regulates fibrinolysis by inhibiting tPA or urokinase.

ASSAY CHARACTERISTICS:

- This monoclonal antibody based assay, has a homogeneous reactivity to the various forms of PAI-1, latent, active, bound to vitronectin, complexed to tPA, or to uPA, or inactive.
- Detection threshold ≤0.5 ng/ml.
- Intra-assay: 3-8%.
- Inter-assay: 5-10%.
- No significant interference of heparin up to 2 IU/ml

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