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ZYMUTEST™ PAI-1 Antigen

REF RK012A-RUO

96 tests

Quantitative assay for the detection of PAI-1 Antigen by ELISA

FOR RESEARCH USE ONLY.

DO NOT USE IN DIAGNOSTIC PROCEDURES.

English, last revision: 11-2022

INTENDED USE:

The ZYMUTEST™ PAI-1 Antigen kit is an ELISA test for quantitative assay of PAI-1 Antigen (Tissue-Plasminogen Activator Inhibitor, Type I), using in human plasma.
This kit is for research use only and must not be used for patient diagnosis or treatment.

SUMMARY AND EXPLANATION:

Technical:
PAI-1 is a single chain glycoprotein, synthesised by endothelial cells and hepatocytes. Its molecular weight is 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.

PRINCIPLE:

First, the immunoconjugate, a mouse monoclonal antibody specific for PAI-1:Ag coupled to horse radish peroxidase (HRP), is introduced into the microwells ELISA plate coated with a second monoclonal antibody specific for PAI-1:Ag. Then, the diluted tested sample is immediately introduced and the immunological reaction starts. When present in the specimen, PAI-1:Ag binds 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 in microwells 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.

REAGENTS:

1. **COAT** **ELISA microplate :** 12x8 contaning 12 strips of 8 wells, coated with monoclonal antibodies, specific of human PAI-1:Ag, then stabilized and packed in an aluminium pouch in presence of a desiccant.
2. **SD FIBRINOLYSIS** **Sample Diluent :** 2 vials of 50 mL, ready to use. Contains Proclin and BSA.
3. **STD** **PAI-1 standard :** 3 vials of 2 mL of PAI-1 Standard, lyophilized. Contains human plasma and BSA.
4. **CI PAI-1** **PAI-1 Plasma Control I High :** 1 vial of 1 mL, lyophilized. Contains human plasma and human PAI-1.
5. **CII PAI-1** **PAI-1 Plasma Control I Low :** 1 vial of 1 mL, lyophilized. Contains human plasma and human PAI-1.
6. **IC** **Immunoconjugate (Anti-(h)-PAI-1-HRP immunoconjugate) :** 3 vials of 4 mL of mouse monoclonal antibodies, specific to the PAI-1:Ag and coupled to peroxidase, lyophilised. Contains BSA.
7. **CD ELISA** **Conjugate diluent :** 1 vial of 25 mL, ready to use. Contains Proclin and BSA.
8. **WS ELISA** **Wash solution :** 1 vial of 50 mL, 20x 20 fold concentrated. Contains Proclin.
9. **TMB** **3,3',5,5'-Tetramethylbenzidine:** 1 vial of 25 mL, ready to use. Contains hydrogen peroxide.
10. **Stop** **0.45M Sulfuric acid:** 1 vial of 6 mL, ready to use.

The standard and controls concentrations may vary slightly from one batch to the next. For the assay, see the exact values provided on the flyer provided with the kit used.

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.
- 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. The strips must be used within 30 minutes.

Reconstitute the contents of each vial with exactly:
STD ➔ **2 mL of** **SD FIBRINOLYSIS** in order to obtain a solution containing about 10 ng/mL of PAI-1:Ag. Shake vigorously until complete dissolution.
CI PAI-1 ➔ **1 mL of distilled water**. Shake vigorously until complete dissolution.
CII PAI-1 ➔ **1 mL of distilled water**. Shake vigorously until complete dissolution.
IC ➔ **4 mL of** **CD ELISA** at least 15 minutes before use. Shake gently until complete dissolution.

SD FIBRINOLYSIS **TMB** **Stop** **CD ELISA**
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.

If the **TMB** substrate becomes yellow, this indicates a contamination. It must be rejected, and a new vial must be used.

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:
STD ➔ **8 hours** at room temperature (18-25°C).
CI PAI-1 **CII PAI-1**
➔ **24 hours** at 2-8°C.
8 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.
IC ➔ **4 weeks** at 2-8°C.
24 hours at room temperature (18-25°C).

Reagent stability after opening, free from any contamination or evaporation, and stored closed, is of:
SD FIBRINOLYSIS
➔ **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 **CD ELISA** **TMB**
➔ **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. EDTA collected human plasma may also be used.
Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI GP44-A4[3] (and CLSI H21-A5[4]) guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references[3,4].

PROCEDURE:

Assay method, one step method:

1. Specimens and controls should be diluted using **SD FIBRINOLYSIS** as described in the table below:

Specimens	Dilution
Controls	**1:5**
Specimens	**1:5**

For expected PAI-1:Ag concentrations > 50 ng/mL, specimens must be diluted at **1:10, 1:20, or more.**

2. Using the standard **STD** with a concentration "C" in ng/mL, prepare the calibration range as described in the table below:

Concentration of PAI-1 (ng/mL)	C	C:2	C:4	C:10	C:20	0
Vol. of **STD**	1 mL	0.5 mL	0.25 mL	0.1 mL	0.05 mL	0 mL
Vol. of **SD FIBRINOLYSIS**	0 mL	0.5 mL	0.75 mL	0.9 mL	0.95 mL	1 mL

Mix gently for homogenization.
The dilutions are stable for **6 hours** at room temperature (18-25°C).

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

Reagent	Volume	Procedure
IC	**100 µL**	Introduce the **IC** in the micro ELISA plate wells.
STD **or CI PAI-1** **or CII PAI-1** **or specimens to test** **or SD FIBRINOLYSIS (blank)**	**100 µL**	Introduce immediately: **STD** **or CI PAI-1** **or CII PAI-1** or **Specimens to test** or **SD FIBRINOLYSIS** into the micro ELISA plate wells
Gently mix either manually or on a microplate shaker. Incubate for 1 hour at room temperature (18-25°C) (a)		
WS ELISA	**300 µL**	Proceed to 5 successive washings **(a)**
TMB	**200 µL**	Immediately, introduce the substrate into the wells. **Nota:** The substrate distribution, raw by raw, must be accurate and at exact time intervals **(b,c)**.
Let the colour develop for exactly **5 minutes** at room temperature (18-25°C) **(c)**		
Stop	**50 µL**	Following exactly the same time intervals, raw to raw, than for the addition of substrate, stop the colour development by introducing the 0.45M sulfuric acid **(b,c)**.
Wait for 10 minutes in order to allow the colour to stabilize then measure absorbance at 450 nm. Subtract the blank values (d).		

(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 **TMB**, the time interval between each row must be accurate and exactly determined.

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

Variant: Tow step method:

The PAI-1:Ag 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 **STD** being reconstituted with **2 mL** of **SD FIBRINOLYSIS**. The **IC** must be reconstituted with **7.5 mL** of **CD ELISA**.

Tested plasma must be assayed at a five fold (**1:5**) dilution or at higher dilutions in **SD FIBRINOLYSIS**, if a concentration higher than 50 ng/mL are expected.

In each microwell ELISA plate, **100 µL** of **SD FIBRINOLYSIS** are introduced, immediately followed by **100 µL** of the calibration solution (prepared as for the one step method) or **100 µL** of the **1:5** diluted tested plasma (specimen or control) (or more is required). Following a **1 hour** incubation at room temperature (18-25°C) and a washing step, **200 µL**/well of **IC** are introduced. Incubate **1 hour** at room temperature, wash the plate, and add the **TMB** (**200 µL**/well). Stop the colour after **5 min** with **50 µL**/well of **Stop** and measure the OD at 450 nm. Washing and operating cautions, as well as results interpretation, are the same as recommended for the one step method.

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.

RESULTS:

- Plot the calibration curve with the OD 450 nm along the Y-axis and the PAI-1 concentration in ng/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.
- For the measurement of PAI-1: Ag concentration, only the calibration curve performed for the series of assays should be used. 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 **CI PAI-1** and **CII PAI-1** the concentrations measured must be multiplied by 5.
- 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, 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:

- 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.

REFERENCES:

1. Declerck P.J. *et al*. Measurement of Plasminogen Activator Inhibitor 1 in biologic fluids with a murine monoclonal antibody based enzyme-linked-immunosorbent assay. Blood. 1998..
2. Declerck PJ *et al*., Multicenter evaluation of commercially available methods for the immunological determination of plasminogen activator inhibitor-1 (PAI-1), Thromb. Haemost. 1993.
3. CLSI Document GP44-A4: "Procedures for the handling and processing of blood specimens for common laboratory tests".
4. 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 ELISA **SD FIBRINOLYSIS** **WS ELISA** H317 : May cause an allergic skin reaction.

Changes compared to the previous version.

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