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ZYMUTEST Rat – PAI-1-Activity

 # RK003A

Rat PAI-1 activity

(Complete ELISA kit for the assay of rat-PAI-1 Activity)

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Last revision: 24/06/2014

INTENDED USE:

The ZYMUTEST Rat PAI-1-Activity kit is a bio-immuno-assay for measuring the rat-PAI-1 (Plasminogen-Activator-Inhibitor-1) activity in rat plasma, or in any fluid where a rat PAI-1 activity can be present.**This kit is for research use only and should not be used for patient diagnosis or treatment.**

ASSAY PRINCIPLE:

ZYMUTEST Rat-PAI-1-Activity is an assay which allows measuring the rat-PAI-1 activity by its capacity to react with tPA. An active tPA complex is coated on the ELISA plate.
The diluted tested plasma or biological fluid is introduced in a microwell. When present, active PAI-1 then binds onto the solid phase, forming tPA-PAI-1 complexes. Following a washing step, the immunoconjugate, a mouse monoclonal antibody (anti rat-PAI-1) coupled to horse radish peroxidase (HRP), is introduced, and binds to a corresponding free epitope of immobilised rat-PAI-1. Following a new washing step, the peroxidase substrate, Tetramethylbenzidine (TMB) in presence of Hydrogen Peroxide, is introduced and a blue colour develops. The colour turns yellow when the reaction is stopped with Sulfuric Acid. The colour developed is directly proportional to the concentration of active rat-PAI-1 in the tested sample

TEST SAMPLE:

- Rat plasma collected on trisodium citrate or Na_2 EDTA anticoagulant.
- Any biological fluid where rat-PAI-1 Activity must be measured.

REAGENTS:

1. **COAT: Micro ELISA plate,** containing 12 strips of 8 wells, coated with an active tPA (recombinant) complex, then stabilised; the plate is packed in an aluminium pouch hermetically sealed in presence of a desiccant.
2. **SD**: 2 vials containing 50 ml of **F-Sample Diluent**, ready to use.
3. **Std**: 3 vials of **active rat-PAI-1 Standard (recombinant)**, lyophilised. When restored with 2 ml of F-Sample diluent, a solution containing "**C"** ng/ml (about 10 ng/ml) of rat-PAI-1 is obtained.
4. **CI:** 1 vial containing **1 ml** of lyophilised **rat PAI-1 Control I** (High).
5. **CII**: 1 vial containing **1 ml** of lyophilised **rat PAI-1 Control II** (Low).

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

6. **IC**: 3 vials of **Anti-(rat)-PAI-1 HRP immunoconjugate**, a mouse 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**. 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 5-300 µl.
- **1-channel pipettes** at variable volumes from 0 to 20 µl, 20 to 200 µl and 100 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. **rat-PAI-1 Activity standard:** restore each vial with **2 ml** F-Sample Diluent in order to obtain a solution containing "**C" ng/ml** rat-PAI-1. This solution **must be used within 1 hour**.
4. **Rat PAI-1 Control I (high):** restore with **1 ml** F-Sample Diluent.
5. **Rat PAI-1 Control II (low):** restore with **1 ml** F-Sample Diluent.

Nota: when restored, **controls I and II must be used within 1 hour**.
Warning: Plasma controls I and II (4&5) and standard (3) contain BSA. Any product of biological origin must be handled with all the required cautions, as being potentially infectious.

6. **Anti-(rat)-PAI-1 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. 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. 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 plasma (9 vol.) must be collected on 0.109M citrate anticoagulant (1 vol.); plasma supernatant is decanted following a 20 min. centrifugation at 2,500 g; citrated plasma should be tested within 2 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 1 hour.
EDTA collected rat plasma may also be used. Conditions of storage are the same than those for citrated plasma.

Calibration:

Using the "C" ng/ml rat PAI-1 activity standard provided in the kit, prepare the following standard solutions:

Active rat-PAI-1 concentration (ng/ml)	C	C/2	C/4	C/10	C/20	0
Vol. of Standard at C ng/ml	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 homogeneisation.
The standard dilutions must be used **within 1 hour** following their preparation.

Tested plasma or sample:

The sample must be tested diluted two fold (**1:2**) in the F-Sample Diluent. For expected PAI-1 concentrations > "2C" (or >20 ng/ml), plasma or samples can be diluted **1:5**, or **1:10**, or **1:20**. For very low expected rat PAI-1 concentrations (< 2 ng/ml) undiluted samples can be used.

Plasma Controls I and II must be tested diluted **two fold (1:2)**, with F-Sample Diluent.

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-(rat)-PAI-1-HRP. (Restored with 4 ml of Conjugate Diluent)	**100 µl**	Introduce the Anti-(rat)-PAI-1- HRP immunoconjugate in the micro ELISA plate wells
Active Rat-PAI-1 Standard or tested sample or controls or F-Sample Diluent (blank)	**100 µl**	Introduce **immediately** the standard solutions or the tested samples in the corresponding micro ELISA plate well (**a**)
Mix gently on a plate shaker or manually and incubate for 2 hours at room temperature (18-25°C)(b)		
Wash Solution (20 fold diluted in distilled water)	**300 µl**	Proceed to 5 successive washings using the washing instrument. **(c)**
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 **(c,d)**.
Incubate for exactly 5 minutes at room temperature (18-25 °C) (b)		
0.45 M Sulfuric Acid (SA)	**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 **(d).**
Wait for **10 minutes** in order to allow the colour to stabilize and measure absorbance at **450 nm (A450)**, within the following hours. Subtract the blank value **(e)**.		

Note:

a) Distribute calibrators, controls and tested specimen as rapidly as possible, in order to obtain an homogeneous immunological kinetics for antigen binding. A too long delay between the distribution of the first and the last wells may induce an influence of immunological kinetics and produce wrong results.

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

c) 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. 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.

d) 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.

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

Two-step procedure:

If necessary, the assay can be sensitized by using a two-step procedure. In this case, the conjugate (**IC**) must be restored with **7.5 ml** of Conjugate Diluent (**CD**). The other reagents are restored as for the one-step method. The calibration curve is **two fold (1:2)** diluted respectively to the one step method (i.e. into each well introduce **100 µl** of F-Sample diluent (**SD**) and **100 µl** of the standard concentration range). The plasma is diluted **two fold (1:2)** directly into the microwell, by introducing **100 µl** of F-Sample Diluent and **100 µl** of plasma (practically, introduce, first, 100 µl of **SD** into each one of the microwells. Then introduce 100 µl of the active rat-PAI-1 standards (**Std**), ranging from 0 to C ng/ml, or 100 µl of undiluted rat plasma). The actual dynamic range then goes from **0 to C/2 ng/ml** (or 0 to about 5ng/ml) for the two-fold diluted standards. The assay is performed using a **2 hours** incubation step for active rat-PAI-1 binding, followed by a washing step, the introduction of immunoconjugate (**IC**) (**200 µl**/well), a new **2 hour** incubation step, another washing step, and finally, the colour development is initiated by the introduction of **TMB/H_2O_2** (200 µl/well) substrate, and stopped after 5 min. with **50 µl**/well of **SA**. The conditions for washing and the cautions are the same than for the one-step method. The measured active rat-PAI-1 concentrations must be multiplied by **2** for correcting for the **two fold (1:2)** dilution factor.

RESULTS:

- On a linear graph paper plot the **rat PAI-1 activity concentrations (ng/ml)** on abscissa and the corresponding absorbances (**A450**) on ordinates.
- Users must construct their own calibration curve, obtained using their standard dilutions (see model on the flyer). From the curve obtained, deduce the rat PAI-1 activity concentration for the tested dilution. For obtaining the rat PAI-1 activity concentration in the tested sample, the value read on the calibration curve must be **multiplied by the dilution factor** (**i.e. 1, 2, 5, 10, 20.....**)
- For controls I and II, the concentrations measured must be multiplied by **2**.
- 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.

Note: The recombinant rat PAI-1 used for preparing the rat PAI-1 Activity standard is only partly active. The "C" ng/ml concentration indicated corresponds to the actual amount of active PAI-1. However, the rat PAI-1 protein, measured with immunoassays (ZYMUTEST rat PAI-1 Antigen) is usually present at a higher concentration (2 to 3 fold higher).

EXPECTED RANGE:

- PAI-1 activity concentration in normal rat plasma is low (< 2 ng/ml).
- In lysed platelet rich rat plasma the PAI-1 Activity concentration is increased.
- In plasma from lipopolysaccharide treated rats, the PAI-1 Activity concentration is dramatically increased.

ASSAY REACTIVITY:

ZYMUTEST rat-PAI-1 Activity only measures active PAI-1, which can form complexes with bound tPA. It is insensitive to inactive or complexed forms of rat-PAI-1.
The assay then only measures the active rat-PAI-1 present in the tested sample.