Rabbit PAI-1 Activity ELISA Kit

Catalog No: IRBPAIKT **Lot No:** SAMPLE

INTENDED USE

Rabbit PAI-1 activity assay is intended for the quantitative determination of active plasminogen activator inhibitor type 1 (PAI-1) in rabbit plasma. **For research use only.**

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BACKGROUND

PAI-1 is involved in the regulation of the blood fibrinolytic system. Increased plasma levels of PAI-1 are implicated in the impairment of fibrinolytic function and may be associated with thrombotic diseases [1,2]. Levels of PAI-1 increase with age [3] and are elevated in conditions such as normal pregnancy [4] and sepsis [5].

ASSAY PRINCIPLE

Functionally active PAI-1 present in plasma will bind to the urokinase (uPA) coated onto a micro titer plate. Latent or complexed PAI-1 will not bind to the plate and will not be detected. After appropriate washing steps, anti-human PAI-1 primary antibody binds to the captured protein. Excess antibody is washed away, and bound antibody is reacted with peroxidase conjugated secondary antibody. TMB substrate is used for color development at 450 nm. Color development is proportional to the concentration of macroglobulin in the samples.

REAGENTS PROVIDED

- **96-well uPA coated microtiter strip plate** (removable wells 8x12) containing uPA, blocked and dried.
- **10X Wash Buffer:** 1 bottle of 50ml
- **Rabbit PAI-1 activity standard:** 1 vial lyophilized standard
- **Anti PAI-1 primary antibody:** 1 vial lyophilized monoclonal antibody
- **Horseradish peroxidase secondary antibody:** 1 vial concentrated HRP labeled antibody
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- **TBS buffer:** 0.1M Tris, 0.15M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water

SAMPLE COLLECTION

Collect 9 volumes of blood in 1 volume of a 3.8% trisodium citrate or acidified citrate. Immediately after collection of blood, samples must be centrifuged at 3000Xg for 15 minutes. It is important to ensure a platelet free preparation since platelets can release PAI-1. The plasma must be stored on ice prior to analysis. The PAI-1 activity samples collected is stable for up to 24 hours or stored at –20°C for up to one month and thawed three times without loss of PAI-1 activity.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard

Reconstitute standard by adding 1ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 50ng/ml standard solution.

Dilution table for preparation of rabbit PAI-1 standard:

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

PAI-1 concentration (ng/ml)	Dilutions
50	100µl from standard vial
25	500µl (BB) + 500µl (50ng/ml)
10	600µl (BB) + 400µl (25ng/ml)
5	500µl (BB) + 500µl (10ng/ml)
2	600µl (BB) + 400µl (5ng/ml)
1	500µl (BB) + 500µl (2ng/ml)
0.5	500µl (BB) + 500µl (1ng/ml)
0.25	500µl (BB) + 500µl (0.5ng/ml)
0.1	600µl (BB) + 400µl (0.25ng/ml)
0.05	500µl (BB) + 500µl (0.1ng/ml)
0	500µl (BB) Zero point to determine background

Standard and Unknown Addition

Remove microtiter plate from bag and add 100µl PAI-1 activity standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures PAI-1 activity levels in the 0.05-50 ng/ml range. If the unknown is thought to have high PAI-1 activity levels, dilutions may be made in blocking buffer.

Primary Antibody Addition

Reconstitute primary antibody by adding 11ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Secondary Antibody Addition

Dilute 3µl of conjugated secondary antibody in 10ml of blocking buffer and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

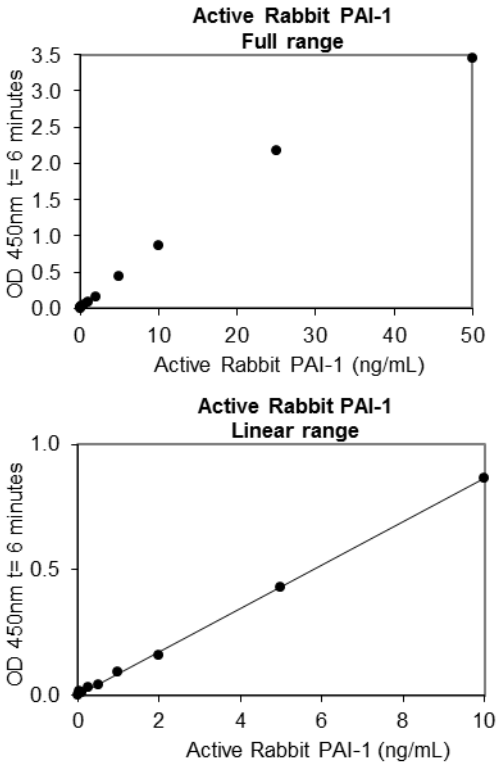
Measurement

Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. For best results read plate immediately. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

Plot A_{450} against the amount of PAI-1 in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of PAI-1 activity in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):



EXPECTED VALUES

The concentration levels in rabbit plasma of PAI-1 activity and PAI-1 antigen were reported to be 9.8±4.6 ng/ml and 20.5±13.5 ng/ml, respectively; the concentration levels in rabbit serum of PAI-1 activity and PAI-1 antigen were reported to be 2.9±2.0 ng/ml and 11.8±4.9 ng/ml, respectively [6]. PAI-1 activity levels have also been reported as ≤ 5 ng/ml [7] and as 3 AU/ml [8] or 4 AU/ml [9], where 1 AU is defined as the amount of plasma PAI-1activity that completely inhibits 1 IU of tPA.

Abnormalities in PAI-1 levels have been reported in the following condition:

- Endotoxemia: Endotoxin induces a time dependent increase in both PAI-1 antigen and activity levels (40- to 90 fold) [6, 10].
- Nitrate treatments: Sodium nitroprusside (NP) is reported to inhibit the release of PAI-1 from platelets [11].
- Hyperinsulinemia: Increased levels of proinsulin and insulin in plasma increase PAI-1 activity levels [7, 12].
- Hypercholesterolemia: High cholesterol diet increased PAI-1 activity levels in rabbit plasma [13].

PERFORMANCE CHARACTERISTICS

Sensitivity These studies are currently in progress. Please contact us for more information.

Intra-assay Precision: These studies are currently in progress. Please contact us for more information.

Inter-assay Precision: These studies are currently in progress. Please contact us for more information.

Recovery: These studies are currently in progress. Please contact us for more information.

Linearity: These studies are currently in progress. Please contact us for more information.

Specificity: These studies are currently in progress. Please contact us for more information

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

REFERENCES

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Example of ELISA Plate Layout

96 Well Plate: 22 Standard wells, 74 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.05 ng/ml	0.1 ng/ml	0.25 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	25 ng/ml	50 ng/ml	
B	0	0.05 ng/ml	0.1 ng/ml	0.25 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	25 ng/ml	50 ng/ml	
C												
D												
E												
F												
G												
H												