

SPECTROLYSE® PAI-1

REF 101201

*a chromogenic assay for measuring
PAI-1 activity in human plasma*



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INTENDED USE

Spectrolyse® PAI-1 is intended for the quantitative determination of Plasminogen Activator Inhibitor Type-1 (PAI-1) activity in human plasma. The test is for *in vitro* diagnostic use and is not intended for internal use in humans or animals.

EXPLANATION OF THE TEST

Plasminogen Activator Inhibitor Type-1 (PAI-1) is a rapid, specific inhibitor of plasminogen activator in plasma.¹⁻³ As a primary regulator of fibrinolysis, PAI-1 is found in a number of different tissues and cell types including macrophages and monocytes, hepatocytes, vascular endothelia cells, adipose tissue of the heart and lungs and in platelets.⁴⁻⁵ The clinical interest in measuring PAI-1 in plasma is based on case studies in which levels of this serine protease inhibitor are associated with various thrombotic and fibrinolytic complications. Deficiency of PAI-1 activity is associated with bleeding disorders wherein the routine hemostatic screening tests are normal.⁶⁻⁷ An increased level of PAI-1 is a key factor in impaired fibrinolytic function and may be associated with thrombotic diseases.⁸⁻⁹ High levels of PAI-1 activity are found in patients suffering from myocardial infarction, hemolytic uremic syndrome, and stroke.¹⁰⁻¹⁴ Levels of PAI-1 in the plasma of pregnant women are also correlated with gestational diabetes, reduced placental blood flow and preeclampsia.¹⁵⁻¹⁸ Patients with cirrhosis may also have elevated levels of PAI-1.¹⁹

PRINCIPLE OF THE METHOD

Spectrolyse PAI-1 is a two-stage, indirect chromogenic assay for measuring PAI-1 levels in plasma as described by Chmielewska *et al.* and Eriksson *et al.*²⁰ In the first stage, a fixed amount of human tPA is added to the plasma sample and allowed to react with the PAI-1 present. Next, the sample is acidified to destroy alpha-2-antiplasmin that would otherwise interfere with the assay. In the second stage, the residual tPA activity is measured by adding the sample to a mixture of human glu-plasminogen, poly-D-lysine and a chromogenic substrate for plasmin (PAR). The residual tPA activity in the plasma sample catalyzes the conversion of plasminogen to plasmin, which in turn hydrolyzes the chromogenic substrate. Poly-D-lysine is present as a stimulator of this tPA catalyzed conversion of plasminogen to plasmin. The amount of color developed is inversely proportional to the amount of PAI-1 activity in the sample. One unit of PAI-1 activity (IU) is defined as the amount of PAI-1 that inhibits one International Unit (IU) of human tPA.

REAGENTS

Spectrolyse PAI-1 contains reagents for 60 assays using a microwell test format.

- R1 1 vial of Imidazole Buffer, 3 mL, 10X concentrated
- R2 2 vials of Plasminogen Activator Reagent (PAR)
- R3 1 vial of 1-chain human tPA, 6000 IU/mL, lyophilized
- R4 1 vial of tPA/PAI-1 Depleted Plasma, 1 mL, lyophilized
- R5 1 vial of Acetate Buffer pH 3.9, 7 mL
- R6 1 vial of Stop Reagent, 4 mL

WARNING

The source material for reagents in this kit have been found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV-2) using FDA Approved methods. As no known test method can provide complete assurance that products derived from human blood will not transmit HBsAg, HCV, HIV-1, HIV-2 or other blood-borne pathogens, this reagent should be handled as recommended for any potentially infectious human specimen.



For *In Vitro* Diagnostic Use. Not for internal use in humans or animals. Do not use the kit components beyond the stated expiration date. Do not mix reagents from different kits. Avoid microbial contamination of the reagents. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled. Do not pipette reagents by mouth. Wear laboratory coat and disposable gloves throughout the test procedure and wash hands thoroughly afterwards. Avoid splashing or aerosol formation.

REAGENT PREPARATION AND STORAGE

Reagents are stable until the expiration date printed on their vial labels when stored at 2°-8°C. Reconstitute and prepare each reagent according to the following instructions.



A. Imidazole Buffer (R1)

Prepare working strength Imidazole Buffer by diluting the 10X concentrate 1:10 with 0.22 µm filtered deionized/distilled water (e.g. add 1 mL of concentrate to 9 mL of water). Working strength Imidazole Buffer is stable for:

	1 month
	2°-8°C



B. Plasminogen Activator Reagent (R2)

Reconstitute a vial with 6.25 mL of working strength Imidazole Buffer. Mix and let stand on ice for 15 minutes. Reconstituted reagent is stable for:

	24 hours
	2°-8°C



C. 1-chain human tPA (R3)

Reconstitute with the volume of Acetate Buffer as specified on the vial label, mix gently and place on ice to obtain a solution containing 6000 IU/mL. Reconstituted tPA may be used for up to 4 days when stored at 2°-8°C. Prepare a 40 IU/mL tPA solution by adding 50µL of tPA to 7.5 mL of working strength Imidazole Buffer. Place this 40 IU/mL solution on ice until using in the assay. The 40 IU/mL tPA solution is stable for:

	24 hours
	2°-8°C



D. tPA/PAI-1 Depleted Plasma (R4)

Reconstitute the vial with 1.0 mL of 0.22 µm deionized/distilled water and gently mix. Allow 15 minutes for complete dissolution. The tPA/PAI-1 Depleted Plasma is stable for:

	6 months
	-20°C



E. Acetate Buffer (R5)

Supplied ready to use. Acetate Buffer is stable for:

	1 year
	2°-8°C

F. Stop Reagent (R6)

Supplied ready to use. Stop Reagent is stable for:

	1 year
	2°-8°C

SPECIMEN COLLECTION AND PREPARATION

Citrate collected platelet poor plasma must be used for this assay. See "Collection, Transport and Processing of Blood Specimens for Testing Plasma-based Coagulation Assays; Approved Guidelines-Fourth Edition", NCCLS Document H21-A4, Vol. 23, No. 35, December 2003.²¹ Plasma collection should be performed as follows:

1. Using a syringe or evacuated siliconized tubes, collect 9 parts of blood into 1 part of 3.2% (0.109 M) trisodium citrate (dihydrate form) anticoagulant solution.
2. Centrifuge the blood sample at a minimum of 5,000 x g for 10 minutes to yield platelet poor plasma. The plasma should have fewer than platelets/µL. Platelets may also be removed by passing the plasma through a 0.22 micron filter.
3. Plasma should be stored at 2°-8°C and assayed within 2 hours. Alternatively, plasma may be stored at -70°C for up to 6 months.
4. Frozen plasma should be thawed rapidly at 37°C. Thawed plasmas should be stored at 2°-8°C and assayed within 2 hours.

Note: Samples taken with minimal stasis from resting subjects should be used. Large diurnal variations of PAI-1 activity have been reported, morning values being about twofold higher than afternoon values.²² This should be taken into consideration when designing clinical studies and routine applications.

PROCEDURE

Materials Provided – See Reagents

Materials Required But Not Provided

96 well round bottom microwell plate
acetate cover sheet for microwell plate
0.22 µm filtered deionized or distilled
tPA/PAI depleted plasma (ADI REF 273)*
pooled normal plasma, plasmas with known PAI-1 levels
50-300 µL eight channel multi-pipette
0-200 µL, 200-1000 µL single pipettes
3 mL, 15 mL and 50 mL plastic conical tubes
microwell plate reader for reading absorbance at 405 nm
37°C water bath
Melting ice

* may be need to dilute and retest samples with PAI-1 level > 30.0 IU/mL

A. Preparation of “40 IU/mL” and “0 IU/mL” PAI-1 Standards

Initial standards are prepared by adding a known amount of tPA, 40 IU/mL, or buffer to the tPA/PAI-1 depleted plasma. Since PAI-1 inhibits tPA, the plasma that is mixed with buffer and contains no tPA is called the “40 IU/mL” PAI-1 Standard. The absence of tPA is equated to having PAI-1 present. The plasma that is mixed with the 40 IU/mL tPA solution is called the “0 IU/mL” PAI-1 Standard. The presence of tPA is equated to having no PAI-1 activity.

By using this convention, the PAI-1 level in the patient plasma may be read directly from the standard curve.

Prepare PAI-1 standards of 0 IU/mL PAI-1 and 40 IU/mL PAI-1 in plastic test tubes as follows. Incubate the 0 IU/mL and 40 IU/mL PAI-1 standards for 15 minutes at 25°C, allowing for the tPA and PAI-1 to react.

“PAI-1” Standard	Volume of tPA/PAI-1 Depleted Plasma	Volume of 40 IU/mL tPA Solution	Volume of Imidazole Buffer
0 IU/mL	50 µL	50 µL	None
40 IU/mL	50 µL	None	50 µL

B. Preparation of Plasma Samples

Dilute each plasma sample to be assayed 1:2 with the 40 IU/mL tPA solution, i.e. 50 µL of plasma sample + 50 µL of tPA solution. Incubate each plasma sample for 15 minutes at 25°C, allowing for the tPA and PAI-1 to react.

C. Incubation of PAI-1 Standards and Plasma Samples

1. Add 100 µL Acetate Buffer and mix.
2. Incubate 20 minutes in 37°C water bath.
3. Add 2.0 mL of filtered deionized and mix.

D. Preparation of Intermediate PAI-1 Standards

Prepare intermediate PAI-1 standards of 10 IU/mL, 20 IU/mL and 30 IU/mL PAI-1 in plastic test tubes as follows:

“PAI-1” Standard	Volume of “40 IU/mL” PAI-1 Standard	Volume of “0 IU/mL” PAI-1 Standard
10 U/mL	25 µL	75 µL
20 U/mL	50 µL	50 µL
30 U/mL	75 µL	25 µL

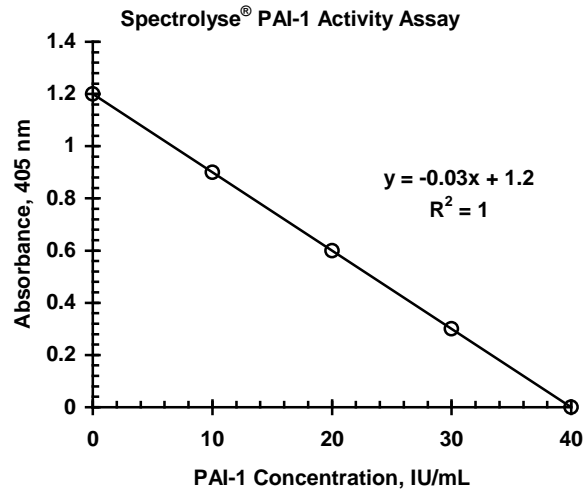
E. PAI-1 Activity Determination

1. Add 20 µL of PAI-1 standard or sample to a microwell.
2. Add 200 µL ice cold PAR to standards and samples.
3. Seal the plate with the acetate cover sheet.
4. Incubate the plate floating in a water bath at 37°C for 90 minutes.
5. Remove from the water bath and add 50 µL Stop Reagent to each microwell.
6. Measure the solution absorbance at 405 nm.

RESULTS

Prepare a calibration curve by plotting the absorbance 405 nm for each PAI-1 standard against its corresponding concentration. A standard curve should be generated each time the assay is performed. The curve shown is for demonstration purposes only.

Representative Standard Curve



CALCULATION

Interpolate the PAI-1 activity of the plasma sample directly from the standard curve. A curve regression software function that is included with the microwell plate reader may be used to calculate the concentrations.

Plasma samples found to contain a PAI-1 level greater than 30.0 IU/mL must be diluted at least 1:4 with tPA/PAI-1 depleted plasma and retested. Then multiply the result by the dilution factor to obtain the PAI-1 concentration of the original plasma sample.

LIMITATION OF PROCEDURE

Discolored plasma samples (e.g., due to hemolysis or increased bilirubin) may give artificially high values. This can be corrected by using the following procedure. In a plastic test tube, mix 500 μ L diluted Imidazole Buffer, 100 μ L Stop and 25 μ L of plasma. Measure the absorbance of both and calculate the difference. Subtract this value from the of the sample to obtain a corrected value to be used for PAI-1 calculations. In practice, the correction procedure is seldom necessary.

EXPECTED VALUES

Plasma samples from 104 healthy adults (57 male, 47 female) were assayed and the data analyzed according to the recommendations set forth in CLSI Document C28-A2.²³ The following PAI-1 values were measured:

Population	PAI-1 Activity, IU/mL		
	Mean	Median	Upper Limit
Adult Male	11.9	9.7	26.8
Adult Female	4.4	7.1	26.2
Total	9.7	7.0	26.7

Each laboratory should establish its own normal reference range from its local population. A minimum of 20 healthy blood donors, including both men and women, spanning the adult age range, should be used. When establishing the normal reference range, collection and preparation of the normal plasma samples must be in the same manner as the plasma samples to be tested. If frozen samples are tested exclusively, then the normal reference ranges should be established using frozen normal samples. It is not recommended to test mixed plasma populations of fresh and frozen samples for establishing the normal reference ranges or for routine testing. The normal reference range should be re-established with each change in reagent lot. The normal reference range data should be obtained over a period of several days to account for day-to-day variations.

QUALITY CONTROL

Control plasmas should be tested with each group of tests run, with a change in personnel or work shift, or according to the testing laboratory's guidelines. A pooled normal plasma and a patient's plasma with a confirmed high level of PAI, aliquoted and stored at -70°C, may be used as controls. The values for the control plasmas should fall within the stated ranges. If the values for the controls exceed the stated ranges, and it has been determined that the equipment is performing properly, the results should be discarded and samples should be rerun with fresh reagents.

TRACEABILITY OF CALIBRATORS AND CONTROL MATERIAL

Information on traceability of calibrators and control material is available upon request.

PERFORMANCE CHARACTERISTICS

Accuracy

A study performed to evaluate SPECTROLYSE PAI-1 against another commercially available PAI-1 activity assay gave a correlation coefficient of 0.953 for 34 samples tested, with a regression equation of $y = 1.0787x - 4.76$.

Precision

The precision of Spectrolyse PAI-1 was determined by assaying three different plasma samples. The samples were each assayed 4 times per run over 20 runs. The results were calculated using duplicate measurements for each plasma (n =40). The intra-assay and inter-assay coefficients of variation (CV) were determined to be as follows:²⁴

Plasma	PAI-1 Activity, IU/mL	n	Intra-Assay CV	Inter-Assay CV
1	10.4	40	3.6%	12.9%
2	13.8	40	3.4%	7.5%
3	22.2	40	2.4%	3.9%

Specificity

The specificity of the assay has been confirmed by assaying plasma samples neat, incubated with an inhibitory monoclonal anti-human PAI-1 IgG and incubated with a non-relevant monoclonal IgG. Incubation with the inhibitory monoclonal anti-human PAI-1 results in either complete quenching or a significant reduction of the PAI-1 activity in the sample, demonstrating that the activity measured is from PAI-1. The results are summarized below.

Sample	PAI-1 Activity, IU/mL				
	Neat	W/Anti-PAI-1 IgG Added	W/Anti-PAI-2 IgG Added	W/Anti-PAI-3 IgG Added	W/Non-Relevant IgG Added
1	29.5	9.3	29.6	29.5	28.7
2	18.2	0	17.2	17.6	9.7
3	35.5	0	35.0	35.0	34.0
4	20.2	0	20.4	19.9	16.3

Sensitivity

The lower limit of detection of the assay has been determined to be 4.6 IU/mL.²⁴

The upper limit of detection of the assay has been determined to be 30.0 IU/mL.²⁴

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