

## Chromolize™ PAI-1

Strip well format. For the quantitative determination of active human plasminogen activator inhibitor, type 1 (PAI-1) in plasma.

For *in vitro* diagnostic use.

**FOR INFORMATION USE ONLY**  
**Not to be used for performing the assay.**  
**Refer to the insert accompanying kit**

### I. INTENDED USE

Biopool Chromolize™ PAI-1 is a bio immunoassay (BIA) for the quantitative determination of active human plasminogen activator inhibitor, type 1 (PAI-1) in human plasma.

### II. SUMMARY

An increased plasma level of PAI-1<sup>(1, 2)</sup> is an important reason for impaired fibrinolytic function and may be associated with thrombotic diseases<sup>(3-5)</sup>. Increased levels of PAI-1 have also been found in conditions such as normal pregnancy<sup>(6)</sup> and sepsis<sup>(7)</sup>.

### III. PRINCIPLE

Chromolize™ PAI-1 is an immunoactivity assay. Active tPA is lyophilized while immobilised on the surface of the microtest well. After reconstitution, plasma samples containing PAI-1 are added and active PAI-1 reacts with the bound tPA. Simultaneously, an HRP-conjugated monoclonal antibody (MA 12A4, see reference 8) to PAI-1 is added. After incubation, unbound sample and conjugated antibody are washed away and the bound PAI-1 quantified with an HRP-sensitive substrate. The amount of colour developed is directly proportional to the concentration of active PAI-1 in the sample.

### IV. DEFINITION OF PAI UNIT

One unit of PAI-1 activity is defined as the amount of PAI-1 that inhibits one international unit of human single chain tPA as calibrated against the International Standard for tPA, lot 86/670 distributed by NIBSC, Holly Hill, London, England. The Chromolize™ PAI-1 assay is standardised against the NIBSC PAI-1 standard 92/654.

### V. REAGENTS

#### A. Reagent description

- 1. Microtest Strips:** 12 tPA-coated, 8-well strips containing human tPA immobilised on the strip well surface.
- 2. PET Buffer:** Phosphate, NaCl, EDTA, and Tween 20 buffer substance sufficient for 1 L solution.
- 3. PAI-1 Standard Plasma, 0 IU/ml:** 4 vials of 0.250 ml lyophilized plasma with 0 IU/ml PAI-1.
- 4. PAI-1 Standard Plasma, 50 IU/ml:** 4 vials of 0.250 ml lyophilized plasma with 50 IU/ml PAI-1.
- 5. Conjugate, 5 ml:** HRP-labelled monoclonal anti-PAI-1 antibody.
- 6. HRP Substrate Solvent, 20 ml:** Phosphate/citrate buffer with hydrogen peroxide.
- 7. HRP Substrate, 5 mg:** 4 tablets containing 5 mg of HRP substrate (1, 2 phenylenediamine dihydrochloride, OPD at 3.33%).
- 8. Reagent Reservoirs:** 6 disposable cardboard trays

### B. Reagent Preparation

- 1. Microtest Strips:** After reconstitution, strips should be used within 15 minutes.
- 2. PET Buffer:** Completely dissolve the contents of the PET Buffer vial in 1 litre purified water (use a magnetic stirrer for about 15 minutes). Use only PET Buffer for conjugate dissolution, strip reconstitution, and washing the strips.
- 3. PAI-1 Standards, 0 IU/ml and 50 IU/ml:** Add 250 µl of purified water to each of the Standard Plasma vials. Gently agitate for 5 minutes to completely dissolve contents before using.
- 4. Conjugate:** Add 5 ml PET buffer directly to the conjugate vial and agitate gently for 5 minutes to completely dissolve contents.

**5. HRP Substrate Solvent and HRP Substrate:** The buffer is ready to use for dissolution of the HRP substrate tablets. Temperature equilibrate required volume (4 ml/tablet) to ambient temperature (20-26°C) before use. Prepare the HRP substrate within 15 minutes before substrate addition. For each 3 strips, dissolve 1 tablet with 4 ml substrate solvent. **NOTE:** It is essential that the substrate solvent is handled with care to avoid contamination that could result in erroneous results.

### VI. STORAGE AND STABILITY

The unreconstituted reagents are stable until the expiration date indicated on the label when stored at 2-8°C

- 1. Microtest Strips:** Store unused strips in a zip-lock bag at 2-8°C and use within one month
- 2. PET Buffer:** Store reconstituted PET Buffer at 2-8°C and use within one month.
- 3. PAI-1 Standards, 0 IU/ml and 50 IU/ml:** Reconstituted PAI-1 standard is stable at 2-8°C for up to 4 hours.
- 4. Conjugate:** Store reconstituted conjugate in the dark at -20°C and use within one month. Thaw by hand warmth before reuse. May be thawed and refrozen 4 times.
- 5. HRP Substrate Solvent and HRP Substrate:** After dissolution: store in the dark at 2-8°C and use within one month.

### VII. WARNINGS AND PRECAUTIONS

The PAI-1 Standard Plasmas are of human origin. Each donor unit of source plasma used in these products has been tested and found negative for Hepatitis B antigens, HIV I and II antibodies, Hepatitis C antibodies, syphilis antibodies and H.T.L.V. I/II antibodies by FDA approved methods. However, no test can offer complete assurance that products derived from human blood will not transmit infectious disease. As with all materials of human origin, this product should be handled as a potentially infectious material. All wastes containing biological material should be properly labelled and stored separately from other wastes. Dispose of all waste materials according to prescribed international, national and local regulations.

The Substrate OPD is harmful and must be handled with care. Avoid ingestion and skin and eye contact. Wear glasses and gloves when handling.

The test should be used in conjunction with clinical observations and results of other laboratory tests.

### VIII. SPECIMEN COLLECTION

Nine volumes of blood are collected in one volume of 0.1 M trisodium citrate. Immediately after blood collection, samples are centrifuged at 3000 X g for 15 minutes. Care must be taken to ensure a "platelet free" preparation, since platelets can release PAI-1. The plasma is transferred to a clean plastic tube and stored on ice prior to analysis.

## IX. LIMITATIONS

Note that large diurnal variations of PAI-1 activity have been reported<sup>(9)</sup>, with morning values being about two-fold higher than afternoon values. This should be taken into consideration when designing clinical studies and routine applications.

## X. PROCEDURE (MANUAL)

### A. Material Provided:

- Micro-Test Strips.
- PET Buffer.
- PAI-1 Standard Plasma, 0 IU/ml.
- PAI-1 Standard Plasma, 50 IU/ml.
- Conjugate.
- HRP Substrate Solvent.
- HRP Substrate.
- Reagent Reservoirs.

### B. Additional Material Required (but not provided):

- 1-channel pipettes covering 0-50 and 200-1000µl.
- 8-channel or repeating pipette for 25-100 µl.
- Purified water (glass distilled or water for injection).
- 1.6 M sulphuric acid.
- Small plastic tubes (2-5 ml) with caps.
- Squeeze bottle.
- Magnetic stirrer and stir bar.
- Paper towels or thin sponge.
- Microtest plate shaker with uniform horizontally circular movement of 3-5 mm.
- Microtest plate spectrophotometer capable of measurements at 492 nm.

**NOTE:** Due to the short incubation times, samples should be added to the wells within 15 minutes. Perform assay steps at ambient temperature.

1. **Preparation of Standard:** Prepare the PAI-1 standard according to the following table.

PAI-1 Concentration (IU/ml)	µl of "0 IU/ml" PAI-1 Standard	µl of "50 IU/ml" PAI-1 Standard
50	0	100
30	40	60
15	70	30
0	100	0

2. **Reconstitution of Microtest Strips:** Add 25 µl PET buffer to each well using a repeating pipette or an 8-channel pipette. Incubate strips on a microtest plate shaker set at a speed of 600-800 r.p.m. for 2 minutes.

### 3. Sample and Conjugate Addition:

- (a) Add 25 µl of plasma samples and PAI-1 Standard to the strip wells. Record positions of samples and standards.
- (b) Add 25 µl Conjugate to the wells using a repeating pipette.
- (c) The time for addition of samples should not exceed 15 minutes, and the time for Conjugate addition should not exceed 1 minute.
- (d) Incubate for 30 minutes on an orbital microtest plate shaker set at a speed of 600-800 r.p.m. It is essential for the result that an efficient shaker is used.

4. **Wash:** Discard the contents and wash the strips four times. Each wash is performed as follows: Fill the wells completely with PET Buffer using a squeeze bottle, if convenient; empty; then "dry" by hitting the plate 4-5 times, face down, against absorbing material (sponge or paper towel). **Important: PET must be used for this step.**

5. **Substrate Incubation:** Add 100 µl HRP substrate to each well. Incubate for exactly 5 minutes on an orbital shaker as above.

6. **Stop:** Stop the reaction by adding 100 µl 1.6 M sulphuric acid to each well and mix. Add in the same order and speed as the substrate was added. If stored in the dark, the coloured product is

stable for at least 2 hours. **Caution! Sulphuric acid is corrosive and must be handled with care. Avoid skin and eye contact. Wear glasses and gloves.**

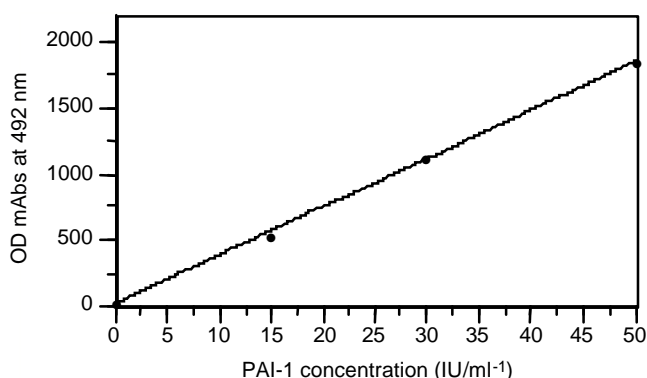
7. **Measurement:** Set the absorbance at 492 nm in a microtest plate spectrophotometer. "Blank" the microtest plate reader against air. Some manufacturers refer to this as the "no reagent blank". Measure the absorbance in all wells at 492 nm, A<sub>492</sub>.

## XI. CALIBRATION.

Plot A<sub>492</sub> against the amount of PAI-1 (0, 15, 30, and 50 IU/ml) in the standards. Fit a straight line through the points by a minimum least squares procedure. The PAI-1 activity in the patient's plasma specimen can be determined by interpolation from the standard curve. Note that the slope of the standard curve can show some variation between assays.

### Sample Calibration Curve

**THE CALIBRATION CURVE BELOW IS A SAMPLE ONLY.** Users must construct a standard curve each time the assay is performed.



## XII. QUALITY CONTROL

It is recommended that the performance of each assay with Chromolize™ PAI-1 is controlled. An aliquoted frozen (-70°C) pooled normal plasma may be used as a normal quality control sample. An abnormal (high PAI-1) control can be prepared by freezing (-70°C) aliquots of a patient's plasma known to contain a high level of PAI-1.

## XIII. EXPECTED VALUES

The plasma concentration of PAI-1 in 149 blood donors (citrate samples collected between 7:00 a.m. and 2:00 p.m.) was determined with the Chromolize™ PAI-1 assay and found to be 5.15 ± 7.13 (SD) IU/ml (median = 2.60 IU/ml). In another study conducted in Northern Sweden using 367 healthy subjects with no pre-screening for serum triglycerides<sup>(10)</sup>, the following PAI-1 levels (IU/ml) were observed:

	Men (20-49y)	Women (20-49y)	All (50-59y)
Mean	8.2 ± 6.2	7.0 ± 5.9	12.8 ± 12.1
Median	6.6	5.9	9.6
Maximum	23.3	18.0	40.3

Abnormalities in PAI-1 levels have been reported in the following diseases and conditions:

- **Venous Thrombosis:** Patients with deep-vein thrombosis have been reported to have elevated PAI-1 levels<sup>(11-13)</sup>.
- **Myocardial Infarction:** Patients with recurrent myocardial infarction (MI) within three years of the first attack had significantly higher PAI-1 levels than those without recurrent attack<sup>(4)</sup>. Also, patients with coronary artery disease have been reported to have increased PAI-1 levels<sup>(14, 15)</sup>. In addition, young survivors of an initial myocardial infarction who have elevated PAI-1 levels have an increased risk for recurrence of MI<sup>(5)</sup>.
- **Surgery:** An increase in preoperative PAI-1 levels has been observed in patients who frequently develop post-operative

thrombosis<sup>(6)</sup>. PAI-1 is an acute phase reactant, increasing rapidly following surgery<sup>(16)</sup>.

- **Endotoxemia:** Endotoxin induces a large increase in PAI-1 levels<sup>(17)</sup>.
- **Pregnancy:** PAI-1 levels increase, as do tPA levels<sup>(3)</sup>.

#### XIV. PERFORMANCE CHARACTERISTICS:

The user should establish product performance characteristics for the specific instrumentation used.

##### A. Precision

For plasma samples ranging from 2-36 IU/ml, the within-run and run-to-run (n = 10) precision for a given device lot were:

PAI-1 Level	Within-Run		Run-to-Run	
	CV %	S.D. (IU/ml)	CV %	S.D. (IU/ml)
2 IU/ml	3.7	0.07	16.9	0.34
22 IU/ml	2.7	0.58	4.6	1.01
36 IU/ml	2.6	1.00	3.6	1.30

##### B. Accuracy

The correlation of the Chromolize™ PAI-1 with Spectrolyse®/ pL PAI was determined by comparing the results of 40 patient samples collected in citrate. The correlation coefficient was 0.9853, with a regression equation of  $y = 1.066x - 2.34101$

##### C. Sensitivity

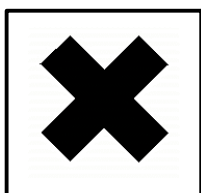
The detection range is 2.0 to 50 IU/ml. Samples giving PAI-1 levels above 50 IU/ml should be diluted in PAI-1 free plasma. The assay is insensitive to PAI-2 concentrations below 1000 IU/ml by virtue of the monoclonal antibody used. In addition, polyclonal PAI-2 antiserum is present in the prefill solution.

##### D. Specificity

The Biopool Chromolize™ PAI-1 Kit measures active PAI-1. Increase of the alpha-2 antiplasmin concentrations by a factor 5 from normal plasma levels (1 μM) has a less than 5% effect on the assay.

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#### XV. RISK AND SAFETY



Xn

##### Harmful – OPD

R20/21/22: Harmful by inhalation, in contact with skin and if Swallowed.

R40: Limited evidence of a carcinogenic effect.

R52/53: Harmful to aquatic organisms, may cause lone-term adverse effects in the aquatic environment.

S36/37/39: Wear suitable protective clothing, gloves, and eye/face protection.

S61: Avoid release to the environment. Refer to special instructions/safety data sheets.

#### XVI. REFERENCES

1. Wiman, B.: Inactivation of tissue plasminogen activator in plasma. Demonstration of a complex with a new rapid inhibitor. *J. Biol. Chem.*, 259:3644-3647, 1984.
2. Kruihof, E.: Demonstration of a fast acting inhibitor of plasminogen activators in human plasma. *Blood*, 64:907-913, 1984.
3. Wiman, B.: The role of the fibrinolytic systems. *J. Lab. Clin. Med.*, 105:265-270, 1985.
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## XVII. KEY GUIDE TO SYMBOLS



Use by



Lot



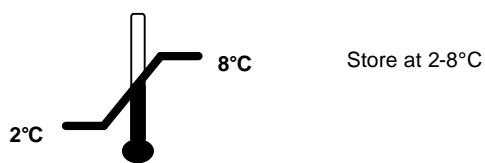
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Manufacturer



For *in vitro* diagnostic use



Store at 2-8°C



Consult accompanying documents



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