

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

Catalog # 210221

## TintElize<sup>®</sup> PAI-1

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

For *in vitro* diagnostic use.

### I. INTENDED USE

Biopool TintElize<sup>®</sup> PAI-1 is an enzyme immunoassay (ELISA) for the quantitative determination of human plasminogen activator inhibitor, type 1 (PAI-1) antigen in human plasma.

### II. SUMMARY

Plasminogen activator inhibitor 1 (PAI-1) is the primary inhibitor of tissue plasminogen activator (tPA), a key enzyme in fibrinolysis. An increased plasma level of PAI-1 is associated with impaired fibrinolytic function. Elevated levels of PAI-1 have been observed in thrombotic disease, acute myocardial infarction, deep vein thrombosis, as well as normal pregnancy and sepsis (1,2). PAI-1 is produced by vascular endothelial cells, platelets, placenta, and kidney tubule cells. PAI-1 is found in several forms: active, inactive (latent) and complexed to tPA and uPA, all of which are measured in the TintElize<sup>®</sup> PAI-1 assay.

### III. PRINCIPLE

The Biopool TintElize<sup>®</sup> PAI-1 test is based on a double antibody principle, similar to the ELISA described by Declerck et al. (1) and uses the same coat antibody (MA-7D4B7). The assay uses quenching and normal antibodies to exclude falsely elevated results from the specific response, a limitation of conventional ELISA assay (2). This method to obtain specific ELISA is "ISAC" (Immunological Specificity and Accuracy Control; patented) (2).

The A-wells (coded green) contain monoclonal antibodies against PAI-1 immobilized on the well surface as well as soluble antibodies against PAI-1. The N-wells (coded blue) contain the same monoclonal antibodies against PAI-1 immobilized on the well surface and soluble non-immune antibodies. During sample incubation, the PAI-1 antigen in the sample is quantitatively bound to antibodies coating the N-wells, but not the A-wells (prevented from binding by the soluble anti-PAI-1 antibodies). HRP-conjugated anti-PAI-1 antibodies are added and will also bind to the PAI-1 molecule. This forms a sandwich of coat antibody: PAI-1: conjugate antibody. The wells are washed to remove excess conjugate and other unbound material. The HRP substrate (OPD/H<sub>2</sub>O<sub>2</sub>) is added and converted to the yellow-coloured product at a rate proportional to the amount of HRP-conjugate bound. The difference in response between the N-well and the A-well is the specific PAI-1 response.

### IV. REAGENTS

#### 1. Microtest Strips:

6 ready to use, 16-well, colour-coded strips coated with mouse anti-PAI-1 monoclonal antibodies and pre-filled with control antibodies.

#### 2. PET Buffer:

PBS-EDTA-Tween 20 buffer concentrate sufficient to make 1 L solution.

#### 3. PAI-1 Depleted Plasma, 0.5 mL:

Lyophilised plasma depleted of PAI-1 by immunoadsorption on a column containing immobilized antibody to human PAI-1. For use with TintElize<sup>®</sup> PAI-1 Assay only.

#### 4. PAI-1 Standard Plasma, 0.5 mL:

Lyophilised human plasma containing 40 ng/mL PAI-1. For use with TintElize<sup>®</sup> PAI-1 Assay only.

#### 5. Conjugate, 7 mL:

Lyophilised goat anti-human PAI-1 IgG conjugated to horseradish peroxidase.

#### 6. Substrate, 2 mL:

Lyophilised *ortho*-phenylenediamine (OPD) with buffer salts.

#### 7. Hydrogen Peroxide, 2 mL:

0.15 % H<sub>2</sub>O<sub>2</sub> in water.

#### 8. Reagent Reservoirs:

6 disposable cardboard trays.

### Reagent Preparation, Storage and Stability

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

- 1. Microtest Strips:** Mark the top and bottom of each strip with indelible ink to prevent mix-ups. Store unused strips in a zip-lock bag at 2-8 °C and use within one month.
- 2. PET Buffer:** Completely dissolve the contents of the PET buffer vial in 1 litre of purified water (use a magnetic stirrer for 10 minutes). Store reconstituted PET buffer at 2-8 °C and use within one month. Use only PET buffer for conjugate dissolution, strip reconstitution, sample dilution, and washing the strips.
- 3. PAI-1 Depleted and Antigen Standard Plasmas:** Add 0.5 mL of purified water to each of the plasma vials and gently agitate for 5 minutes. Store reconstituted reagents capped at 2-8 °C and use within one month.
- 4. Conjugate:** Add 7 mL PET Buffer directly to the conjugate vial and agitate gently for 3 minutes. Store reconstituted conjugate at 2-8 °C and use within one month.
- 5. Substrate:** Dissolve in 2.0 mL of purified water (agitate 10-15 minutes) to prepare substrate concentrate. Freeze excess reconstituted substrate within 30 minutes, store at -20°C and use within one month. May be thawed and refrozen.
- 6. Hydrogen Peroxide:** Transfer to a clean test tube with cap. Store in the dark at 2-8 °C and use within one month.

### Caution and Warning

The PAI-1 Depleted and PAI-1 Standard Plasmas are of human origin. Each unit of source plasma used in the preparation of these products has been tested and found negative for HBsAg, HIV I & II, anti-HBc and anti-HCV 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.

The Substrate OPD and Hydrogen Peroxide are toxic and must be handled with care. Avoid ingestion, skin and eye contact. Wear glasses and gloves when handling.

### V. SPECIMEN COLLECTION

Nine volumes of blood are collected in 1 volume of 0.1 M trisodium citrate or 99 volumes of blood in 1 volume of 0.5 M EDTA, followed by centrifugation at 2500 x g for 15 minutes. See NCCLS Standards (3). Care must be taken to ensure a "platelet free" preparation since platelets can release PAI-1 (4). During collection, only 1/3 of the plasma supernatant should be harvested by using a plastic pipette inserted to half of the plasma depth. Plasma samples should be stored at 2-8 °C and assayed within 2 hours. Alternatively, plasma may be stored at -20 °C for 1 month and thawed once at 37 °C, 30 minutes before use.

### VI. PROCEDURE (MANUAL)

#### A. Material Provided:

Microtest Strips  
PET Buffer  
PAI-1 Depleted Plasma  
PAI-1 Standard Plasma  
Conjugate  
Substrate  
Hydrogen Peroxide  
Reagent Reservoirs

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

8-channel or repeating pipette for 50 - 200 µL  
Pipettes; 5-1000 µL, and 10 mL  
Purified water (deionized or distilled)  
3M sulphuric acid (STOP solution)  
Test tubes: 2-5 mL  
1000 mL graduated glass cylinder  
Squeeze bottle  
Magnetic stirrer and stir bar  
Paper towels or thin sponge  
Horizontal microtest plate shaker  
Microtest plate spectrophotometer capable of measurements at 492 nm  
2 levels of quality control material are recommended (see section VIII).

Perform assay steps at ambient temperature (20-26 °C). Temperature equilibrate all reagent solutions.

**1. Reconstitution of Microtest Plate Wells:** Add 100 µL of PET Buffer to each well using a repeating pipette or an 8-channel pipette. Take care not to contaminate the N-wells (blue colour) with the contents of the A-wells (green colour). Agitate gently for 1 minute. *Please note:* In this step, and when adding standards, samples, and conjugate, work from blue well to green well. Discard the tip after it has been in contact with the green solution. Contamination of an N-well with A-well contents will result in underestimation of PAI-1.

**2. Sample and Standard Addition:** Mix the PAI-1 standard plasma and the PAI-1 depleted plasma in small test tubes as below.

PAI-1 Conc.	PAI-1 Standard (µL)	PAI-1 Depleted Plasma (µL)
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40 ng/mL	200	0
20 ng/mL	100	100
10 ng/mL	50	150
0 ng/mL	0	200

Store tightly capped test tubes at 2-8 °C and use within one month. Add 20 µL of each PAI-1 standard and sample to an N-well and to the adjacent A-well. Record the positions. A colour shift will take place in the wells shortly after addition of plasma samples indicating that an addition has been made.

**3. Conjugate Addition and Incubation:** Add 50 µL of conjugate solution to all wells, incubate the strips in the frame with cover, and agitate on a dedicated microtest plate shaker for 2 hours. A suitable speed is 500-600 r.p.m. at a gyration of 5 mm. (Gel rockers or destainers are not suitable.)

**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:** Prepare OPD/ H<sub>2</sub>O<sub>2</sub> substrate no more than 30 minutes before use. Mix 300 µL substrate concentrate with 3 mL water and add 300 µL H<sub>2</sub>O<sub>2</sub>. If all strips are to be used immediately, add 22 mL of water to the substrate vial and agitate for 15 minutes, then add the full contents of H<sub>2</sub>O<sub>2</sub> reagent. *Note:* The substrate is light sensitive and should be sheltered from excessive light. **Caution!** See substrate warning above. Add 200 µL substrate to each well, using the 8-channel or repeating pipette. Incubate the plate for 15 minutes on a microtest plate shaker at room temperature.

**6. Stop the Reaction:** Add 50 µL STOP solution to each well using the 8-channel pipette. Add the STOP solution with the same speed and order as the substrate was added. Store the plate for 10 minutes in the dark to allow colour stabilisation before measurement. The yellow-coloured product is stable in the dark for 2 hours. **Caution!** Sulphuric acid is corrosive and must be handled with care. Avoid skin and eye contact. Wear glasses and gloves.

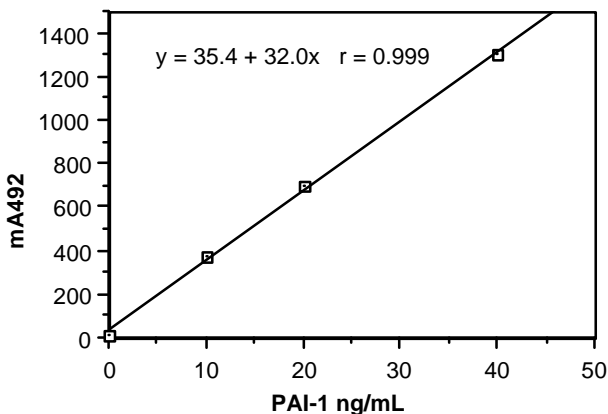
**7. Measurement:** Set the absorbance at 492 nm in a microtest plate spectrophotometer. "Blank" the microtest plate reader against air. Some manufacturers refer to this as the "no reagent blank". Measure the absorbance in all wells at 492 nm, A<sub>492</sub>. Calculate the difference in absorbance (ΔA) between the "N"-value and the corresponding "A"-value for all standard samples and test samples.

#### VII. CALIBRATION:

Plot ΔA against the amount of PAI-1 in the standards. Fit a straight line to the points by a minimum least squares procedure. The PAI-1 antigen 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.



#### VIII. QUALITY CONTROL

It is recommended that 2 plasma samples be stored at -20 °C in small aliquots and used as quality controls each time the assay is performed. These should consist of a high control (30-40 ng/mL) and a low control (5-10 ng/mL). Failure to obtain a PAI-1 level within 2 standard deviations of the mean for each control standard may invalidate the assay. The PAI-1 Standard Plasma and PAI-1 Depleted Plasma which are included in the kit, are necessary to construct the standard curve and must not be used to check kit performance.

#### IX. LIMITATIONS AND INTERFERENCES

1. Use the purest water available. Bacterial and metal ion contamination act as peroxides increasing the background.
2. Allow all solutions to equilibrate to room temperature to avoid anomalous results in the outer wells, referred to as "edge effects".
3. Be sure that the wash volume completely fills the wells and that the wells are completely emptied after each wash. Do not let the wells dry out.

4. Avoid bubbles during the PET wash. Gently squirt extra PET buffer into wells with bubbles and rinse all bubbles away. Just before measurement, carefully wipe the under surface of the plate with a clean, slightly damp cloth to remove salts, dust and other build-up.
5. If the samples contain azide (a peroxidase inhibitor) it is necessary to first incubate the samples for one hour without conjugate, then wash the plate and refill the wells with 100 µL of PET-buffer before adding the conjugate.
6. To assure accurate, reproducible results, use accurate pipetting devices and observe recommended procedures with emphasis on incubation times and temperature.
7. During the preparation of plasma, contamination by platelets should be avoided since platelets contain large amounts of PAI-1 antigen. Only platelet-poor plasma should be frozen and thawed.
8. PAI-1 levels show diurnal fluctuation, with the lowest level at 15:00 (5). Thus, the time of blood sample collection should be standardised.

#### X. EXPECTED VALUES

Normal values of PAI-1 antigen in human platelet-poor plasma have been reported in the range 4-43 ng/mL (18 ±10 ng/mL with a correlation to PAI-1 activity of r=0.80 (1). Patients with recurrent deep venous thrombosis had higher PAI-1 antigen levels: 44 ± 20 ng/mL (1). The PAI-1 antigen levels rise 3- to 6-fold during the third trimester of normal pregnancy (5). This increase is more pronounced in pre-eclamptic pregnancies (6). The time of blood sample collection should be standardised since PAI-1 levels show diurnal fluctuation, with the lowest levels at 15:00 (7).

#### XI. PERFORMANCE CHARACTERISTICS

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

##### A. Precision

The within assay (n=20) coefficient of variation (CV) is 2.9% (S.D.=1.11) at 40 ng/mL and 1.9 % (S.D.=0.40) at 20 ng/mL. The corresponding between assay (n=10) CVs are 3.3 % (S.D.=1.16) and 2.4 % (S.D.=0.49), respectively.

##### B. Accuracy

The accuracy was shown in a study which compared the PAI-1 level of 78 samples using the Biopool Spectrolyse<sup>®</sup>/pL PAI kit (a functional chromogenic assay for PAI-1). In this study, various concentrations of active PAI-1 were added to PAI-depleted plasma. The coefficient of correlation was 0.982, with a regression equation of y = 0.59 x + 3.67.

##### C. Sensitivity

The detection limit is 0.5 ng/mL PAI-1 when the assay is performed according to protocol.

##### D. Specificity

The Biopool TintElize<sup>®</sup> PAI-1 kit measures human plasminogen activator inhibitor 1, endothelial type. It detects active and inactive (latent) forms of PAI-1, as well as that complexed as tPA/PAI and uPA/PAI. The PAI-1 standard has been calibrated against a purified latent PAI-1 standard that has been quantified by amino acid analysis.

#### XII. REFERENCES

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