# TECHNOZYM® t-PA/PAI-1 Complex ELISA
## For research use only REF TC12080

Plasminogen Activator Inhibitor Type I (PAI-1) is the major inhibitor of tissue-type plasminogen activator (t-PA). In the resting state, the body's plasma concentration of PAI-1 greatly exceeds the t-PA plasma level and even following physical exertion or venous occlusion, conditions which lead to increased t-PA levels, PAI-1 is usually available in adequate amounts to compensate for increased activator levels. Thus, increases in t-PA-PAI-1 complexes as long as active, non-complexed PAI-1 is available.

Certain pathological conditions are associated with increased t-PA values. The liver is the major site of t-PA degradation and hepatic disorders are often accompanied by increased t-PA levels and, thus, increased t-PA-PAI-1 complex values. In healthy persons, the t-PA level increases with age and complex levels rise accordingly.

The quantitation of t-PA-PAI-1 complexes aids in the interpretation of alterations in the levels of t-PA and/or PAI-1. Since the liver, in addition to being a site of t-PA degradation, is an organ of PAI-1 synthesis the quantitation of activator-inhibitor complexes may serve as and indicator of the extent to which active PAI-1 is available to compensate for a reduction in the hepatic degradation of t-PA, i.e. elevation of t-PA plasma values.

The determination of elevated t-PA-PAI-1 complex values may be useful for detection of liver dysfunctions.

## Application

The TECHNOZYM® t-PA-PAI-1 complex ELISA is useful for researching elevated amounts of t-PA-PAI-1 complex in subjects with liver disease.

## Test principle

The TECHNOZYM® t-PA-PAI-1 test is a solid phase enzyme immunoassay.

## Specificity

This test system measures t-PA-PAI-1 complexes only. Free t-PA or PAI-1 are not measure nor are other complexes which include these molecule detected. The test measures from 0 ng/mL- 20 ng/mL. The inter- and intra-assay variations are less than 10 % and 5 %, respectively.

## Test samples

Use fresh EDTA plasma samples. Commercially available tubes can be used. After filling, samples should be gently mixed by inverting the tube 5 times, and, then are placed in a crushed ice-water mixture. Centrifuge the blood within 90 min. after the puncture at 2000 g for 30 min at 4 °C (preferably in a cold centrifuge with swing out rotor). Immediately after centrifugation, plasma should be carefully pipetted off and collected in a small plastic tube. After carefully mixing of the pooled plasma, precooled and prelabelled plastic tubes for storage are filled. The freezing operation is very important: snap-freezing, that means almost instantaneous freezing, is highly recommended. Storage temperature should be kept constantly below below –30 °C, and preferably at –70 °C. The total time between blood collection and plasma freezing should not exceed 90 min. Thawing and refreezing of plasma aliquots is not recommended.

## Kit Components:

Determination: 42 sample in duplicate

1. **PLATE + PLATE COVER**
   12x8 well microtiter strips precoated with a monoclonal anti t-PA coating antibody in bicarbonate buffer and blocked with 1% bovine serum albumin (BSA) (TC-Code GN).
2. **STANDARD**
   1 x lyophilised t-PA-PAI-1 complexes (TC-Code BT)
   Concentrations are lot-dependent; consult value table.
3. **POX-ANTIBODY**
   1 x conjugated monoclonal anti-PAI-1 antibody (concentrated) (TC-Code KL)
4. **DILUTION BUFFER – (white cap)**
   1 x 20 mL 2.5 x concentrated (PBS, 1% BSA, 5mM EDTA (TC-Code AD).
5. **POX DILUTION BUFFER – (white cap)**
   1 x 12 mL PBS, 1% BSA (TC-Code DD).
6. **SUBSTRATE - (green cap)**
   1 x 12 mL TMB (Tetramethylbenzidine) in substrate buffer containing $H_2O_2$. Ready to use. (TC-Code KN)
7. **STOP SOLUTION**
   1 x 15 mL sulfuric acid 0.45 mol/l (TC-Code KK)
8. **WASH BUFFER – (blue cap)**
   1 x 20 mL 12x5 x concentrated (PBS 0.5 %, Tween 20) (TC-Code BE)

Kit storage: Store all components at 2-8 °C.

Also required:

1. Micropipettes and a multichannel micropipette; pipette tips
2. Glass or plastic test tubes for diluting the standards.
3. Laboratory bottles or beakers and graduated cylinders for diluting wash and dilution buffer
4. Distilled or deionised water
5. Absorbent paper towels
6. Microtiter plate washer (alternatively, washing can be performed manually using a multichannel pipette or repeating syringe).
7. A microtiter reader equipped with a 450 nm filter and, if possible, a 620 nm reference filter.
8. A 37 °C incubator
9. Graph paper

## Reagent handling and bench stability

All reagents must be at ambient temperature before use.

| Buffer/Standard | Volume/bottle | Additions | Bench/Stability |
|---|---|---|---|
| Dilution buffer | 20 mL | 30 mL distilled water | 4-8 weeks at 4 °C |
| Wash buffer | 20 mL | 230mL distilled water | 4-8 weeks at 4 °C |
| POX AB (concentrate) | - | 1 +50 with POX dilution buffer | Working solution RT: 4 hours |
| | | undiluted | After opening 6 months |
| Standard | - | 0.5 mL distilled water | Aliquot –20 °C 8 weeks RT: 4 hours |

RT = Room Temperature
POX AB = Peroxidase conjugated Antibody

PLATE: Unused strips reseal in aluminium foil bag with desiccant. Store at 4 °C for up to 8 weeks.

**For 8 test wells:**
**Mix    20 µL conjugate with**
**1000 µL POX dilution buffer**

## STANDARD CURVE
Preparation of calibrators for standard curve see value table.

## SAMPLE DILUTIONS

Dilute the plasma samples 1:4 with dilution buffer 1:4 100 µL plasma + 300 µL dilution buffer. If testing samples in which abnormally high complex values are expected, dilute the plasma further i.e. 1:8 or 1:16
1:8  50 µL plasma + 350 µL dilution buffer
1:16 25 µL plasma + 375 µL dilution buffer

## Assay Procedure

1. **SAMPLE/STANDARD ADDITION:**
   Pipette 0.1 mL of the diluted samples/standard into separate wells. Blank well is filled with 0.1 mL dilution buffer. Running standard/sample in duplicate is recommended.
2. **SAMPLE INCUBATION**
   Cover the plate with a plastic foil and incubate for 1hour at 37°C
3. **WASH PLATE**
   Add 0.25 mL of wash buffer to the wells and tip out the contents. Wash the strips twice further with wash buffer. Tap strips on absorbant paper and make sure the wells are completely dry.
4. **POX ANTIBODY ADDITION**
   Add 0.1 mL of the diluted POX anti-PAI-1 antibody to all wells, preferably with a multichannel pipette.
5. **POX ANTIBODY INCUBATION**
   Cover and incubate the plate for 1 hour at 37 °C
6. **WASH PLATE**
   Wash three times as described in step 3.

7. **SUBSTRATE**
   Pipette 0.1 mL of TMB substrate to all wells. Incubate for 25 minutes at room temperature.
8. **STOP**
   Pipette 0.1 mL of stop solution to all wells.
9. **READ**
   Measure absorbance at 450 nm (with 620 nm reference filter if available). Read absorbance within one hour after the addition of the stop solution.
10. **GRAPH**
    Construct a graph of standard curve.
11. Locate the absorbance for each sample on the curve and read the corresponding value from the horizontal axis. <u>Do not forget to multiply by the dilution factor (4, 8 or 16) for the samples.</u>

## t-PA-PAI COMPLEXES STANDARD CURVE
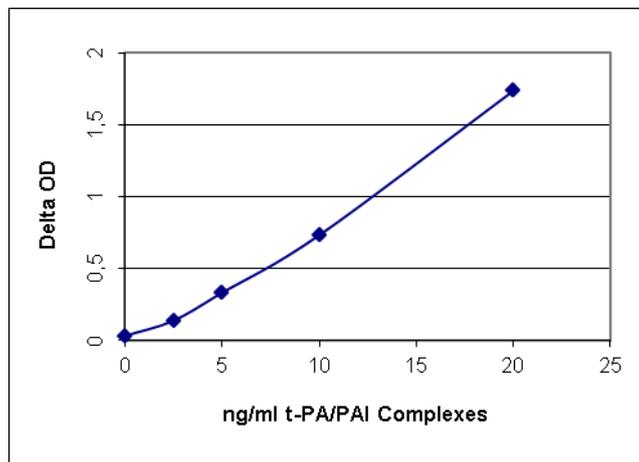


| Time table<br>Summary of procedure | | *time required* | *Temp.* |
|---|---|---|---|
| Reagent, standard sample handling | | 1-2 hours | |
| **1. Sample – incubation** | 100 µL | 1 hour | 37 °C |
| wash 3 times | 250 µL | | |
| **2. POX-AB – incubation** | 100 µL | 1 hour | 37 °C |
| wash 3 times | 250 µL | | |
| **3. Substrate – incubation** | 100 µL | 25 minutes | RT |
| **Stop solution** | 100 µL | | |
| **Read absorbances** | | | |
| **Time total: min.** | | **3-4½ hours** | |

### Evaluation of results

Normal plasma range for t-PA-PAI-1 complex is 7-20 ng/mL.

### Notes

Be sure to prepare all reagents before proceeding with the assay. It is critical to keep the time necessary for pipetting standards and samples to a minimum and avoid delays.
Be sure to wash the plate thoroughly and completely remove any residual wash buffer after each wash cycle. Insufficient washing can lead to erroneously high values and incomplete removal of wash buffer to irregularities due to the dilution of added reagents.
As mentioned use a multichannel pipette to add peroxidase conjugate, TMB substrate and stop solution.

### Warning

Potentially biohazardous material. Donor plasma used in this kit was tested by internationally approved methods for the presence of antibodies to HIV and hepatitis B virus and found to be negative. However, all human blood products should be handled as potentially infectious material.

### Literature

1. C.M. Hekman, D.J. Loskutoff: Fibrinolytic pathways and the endothelium. Seminars in Thrombosis and Haemostasis 13 (4): 514-527, 1987
2. D.J. Loskutoff: Type 1 Plasminogen Activator Inhibitor and its potential influence on thrombolytic therapy. Seminars in Thrombosis and Hemostasis 14 (1): 100-109, 1988
3. L.A. Erickson, C.M. Hekman, D.J. Loskutoff: The primary plasminogen-activator inhibitors in endothelial cells, plateles, serum, and plasma are immunologically related. Proc. Natl. Acad. Sci. USA 82: 8710-8714, 1985