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# TECHNOZYM® PAI-1 Antigen ELISA
# For research use only

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|---|---|---|---|
| REF | TC12075 | TECHNOZYM® PAI-1 Antigen ELISA | Σ 96 |
| REF | TC12077 | TECHNOZYM® PAI-1 Antigen Calibrator Set | 5 x 0,5 ml |
| REF | TC12079 | TECHNOZYM® PAI-1 Antigen Control Set | 2 x 0,5 ml |

**Symbols key**

| | | | |
|---|---|---|---|
| | Manufacturer | | Expiry date |
| | Storage temperature | | Consult instructions for use |
| AQUA | Distilled water | Σ | Determinations |
| BUF | Reaction buffer | LOT | Lot |
| CAL | Calibrator | MTP | Microtiter plate |
| CONJ | Conjugate | REF | Catalogue number |
| CONT | Control | RTU | Ready to use |
| DIL | Dilute or dissolve in | STOP | Stop solution |
| INC | Incubation buffer | SUB | Substrate |
| RUO | For research use only | WASH | Washing solution concentrate |
| | | | |

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# TECHNOZYM® PAI-1 Antigen ELISA

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## PRODUCT DESCRIPTION

### INTENDED USE

The TECHNOZYM® PAI-1-antigen-ELISA can be used to determine PAI-1 antigen levels in samples with thrombotic disorders (deep vein thrombosis, myocardial infarction, stroke), malignancies or septicaemia.

### COMPOSITION

1. ELISA test strips (12), with 8 wells each, coated anti PAI-1 monoclonal antibody; the drying agent is supplied in an aluminium bag.
2. Washing buffer concentrate (PBS; pH 7.3); containing detergent; 0.01% merthiolate; 1 bottle, 80 mL.
3. Incubation buffer (PBS; pH 7.3); contains stabiliser protein; 0.05% proclin and dye, 1 bottle, 90 mL, ready for use.
4. Calibrators (Standards) numbered; lyophilised; 1 bottle each. **Concentrations are lot-dependent; consult label on the vial.**
5. Control plasmas "low level" and "high level" for checking purposes lyophilised; 1 bottle each. **Concentrations are lot-dependent; consult the label on the vial.**
6. Conjugate monoclonal Anti-PAI-1-POX; dyed blue; 1 bottle, 0.3 mL.
7. Chromogen TMB (tetramethylbenzidine); 1 bottle, 12 mL; ready for use.
8. Stopping solution sulphuric acid 0.45 mol/L; 1 bottle 12 mL; ready for use.
9. Adhesive film: for ELISA test strips (2).

### MATERIAL REQUIRED (but not supplied with the kit)

1. Distilled water
2. Test tubes for diluting standard and samples
3. Measuring cylinder (1000 mLl)
4. Precision pipettes (10, 100 and 1000 µL)
5. Variable pipette (1000 µL)
6. Multichannel and/or dispensing pipettes (100 and 200 µL)
7. ELISA washer or multichannel pipette
8. ELISA reader with 450 nm filter, with a 620 nm reference filter if available.
9. Incubator (+37°C)

### WARNING AND PRECAUTIONS

- For research use only
- All human blood or plasma products as well as samples must be considered as potentially infectious. They have to be handled with appropriate care and in strict observance of safety regulations. The rules pertaining to disposal are the same as applied to disposing hospital waste.
- Calibrators and control plasmas made from human blood and any individual plasma involved in the procedure is $HB_s$ Ag, HIV 1/" Ab and HCV-Ab-negative (see labels on kit and/or bottles).
- Stopping solution (sulphuric acid) may irritate the skin. Should acid get into your eyes, wash out immediately with water and consult a doctor.
- The reagents sometimes contain preserving agents (merthiolate). Beware of swallowing! Avoid contact with skin or mucous membranes

## STABILITY AND STORAGE

The expiry date printed on the labels applies to storage of the unopened bottles at +2…8°C.

Stability after reconstitution/opening:

| Material/Reagent | State | Storage | Stability |
|---|---|---|---|
| Calibrators, control plasmas | after reconstitution | -20°C | 6 months |
| ELISA test strip | after opening | +2…8°C with adhesive film in plastic bag with drying agent | expiry date |
| Washing buffer concentrate | after opening | +2...8°C | 6 months |
| Washing buffer | 1+11.5 dilution of concentrate | +2...8°C | 3 weeks |
| Incubation buffer | after opening | +2...8°C | 2 months |
| Conjugate | after opening | +2...8°C | 6 months |
| | working solution | room temperature +18…25°C | 60 minutes |
| Chromogen TMB | after opening | +2...8°C | expiry date |

## TEST PROCEDURE

### PREPARATION OF SAMPLES

Sample material: Plasma.
It is highly recommended to use commercially available collecting tubes which contain platelet stabilizing agents e.g. CTAD. 90% of PAI-1 antigen is contained in the platelets so it is essential to ensure during sample collection that the platelets are not damaged which would result in elevated plasma levels. Citrated or EDTA plasmas can be used. Centrifuge for 15 minutes at a minimum of 2500 g (DIN 58905). The plasma sample may be stored for 3 hours at room temperature; otherwise the sample ought to be frozen immediately after centrifugation at –20°C or below. Plasmas are stable for 6 months at –20°C.
Serum samples should not be used as they show high PAI-1 levels which are related to the platelet PAI-1 content. Cell supernatants and tumour extracts can be used, but this ELISA test has been optimized for plasma samples, therefore other dilution factors would have to be used accordingly.

### PREPARATION OF REAGENTS

1. Before starting the test, all the required components are to be brought to room temperature.
2. Preparing the washing buffer: Dilute 1 part by volume washing buffer concentrate with 11.5 parts by volume distilled water (1+11.5). Mix well! (Diluted washing buffer concentrate = washing buffer). There may be crystalline precipitations which will dissolve at 37°C within 10 minutes.
3. Label/number strips with a water resistant pen in case the strips accidentally fall out of the frame during testing.
4. Reconstituting calibrators and control plasmas: Calibrators and control plasmas are reconstituted with 500 µl distilled water and mixed for 10 seconds after a reconstitution time of 15 minutes (vortex mixer). Reconstituted components are clear to slightly turbid.
5. Preparing the conjugate working solution (1+50):
   Dilute 1 part by volume conjugate with 50 parts by volume incubation buffer.

**For 8 test wells: Mix 20 µl conjugate with 1000 µL incubation buffer**

### PERFORMANCE OF THE TEST

| | | |
|---|---|---|
| **SAMPLE INCUBATION** (reference 1,2) | **Calibrators, control plasmas and samples** into test wells, Add incubation buffer to all wells cover test strips with film | **25 µL 75 µL** |
| | incubate at +**37°C** | **60 minutes** |
| **CONJUGATE REACTION** (reference1,2) | empty wells thoroughly and pipette **conjugate working solution** into wells, cover test strip with film | **100 µL** |
| | incubate at +**37°C** | **60 minutes** |
| **WASHING **** (reference 1,3,4) | **washing buffer** | **3 x 200 µL** |
| **SUBSTRATE REACTION** (reference1,2) | pipette **substrate solution** into test wells, cover test strip with film | **100 µL** |
| | incubate at **room temperature** (+18…25°C) | **10 minutes** |
| **STOPPING** (reference 1,2) | pipette **stopping solution** into wells | **100 µL** |
| **MEASURING** (reference 5) | ELISA-Reader, **450 nm** | shake 10 sec., measure within 10 minutes |

**In use of an ELISA vending machine or automatic ELISA Washers wash please 6x.

References
1. Reagents of different lots must not be combined
2. Precision and performance, among others, essentially depend on the following factors:
- Thorough mixing of all substances used for dilution
- Test calibrators, controls and samples in duplicates.
- Incubation to be done at correct temperatures
- Strict observance of the order of pipetting and of the time element as indicated:
- The time for sample incubation, conjugate and substrate reaction as indicated starts after pipetting the last sample. Incubation times should not vary by more than ±10%.
- During sample incubation and conjugate reaction, the time for pipetting the diluted calibrators/samples/control plasmas and/or conjugate solutions must not exceed 60 seconds per ELISA test strip (8 wells).
- During substrate reaction and at stopping, the time needed for pipetting the substrate and/or the stopping solution must not exceed 10 seconds per ELISA test strip. Short pipetting times may be secured by using multichannel- and dispensing pipettes.

**3. After the last washing, wells must be aspirated thoroughly, turned upside down and positioned on a blotting paper; by gentle tapping, the last remnants must be removed.**

4. Measuring the difference in wave lengths at 450 and 620 nm or at 450 and 690 nm, the precision of the test is increased.

### LIMITATION OF THE TEST

Samples which fall higher than the top calibrator standard must be retested at a higher dilution as a hook dose response occurs above 130 ng/mL.

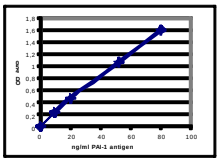
## ANALYSIS RESULTS

### CALCULATION OF THE RESULTS

Setting up a reference curve:

Example of standard curve.

X axis: Concentration PAI-1 antigen ng/ml
Y axis: Extinction
Graph plot is linear-linear.




Assessment of reference curve
- The extinction coefficient of the highest calibrator should be between 1.0 and 2.5.
- The validity of the test may be checked on the basis of the calculated control values.

Measuring concentration of samples
- Read off the concentration from the reference curve.
- If there are samples with extinction coefficients higher than that of the highest point on the curve, they have to be prediluted with incubation buffer (1+1). The measured concentration then has to be multiplied with the dilution factor 2.

### REFERENCE RANGE

Normal range for PAI-1 antigen is between 7 – 43 ng/mL (n = 32 citrated plasmas)
It is recommended that individual laboratories establish their own normal range. PAI-1 antigen levels above 100 ng/mL may indicate reduced fibrinolytic capacity and thus increased thrombotic tendency. PAI-1 is an acute phase protein and its plasma concentration increases in conditions where increased Interleukin I levels are observed, e.g. infections, some malignancies and during the postoperative period. Elevated PAI-1 levels are also associated with myocardial infarction and coronary artery disease. This assay measures free, complexed and latent PAI-1 and is not affected by other plasminogen activator inhibitors.

### PERFORMANCE CHARACTERISTICS

Performance data are given below. Results obtained in individual laboratories may differ.

### PRECISION

Reproducibility was determined with different samples (in series and day to day). The following results were obtained.

| | Intra assay variation | | Inter assay variation | |
|---|---|---|---|---|
| Sample | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
| N | 12 | 12 | 6 | 6 |
| Mean (ng/mL) | 42.70 | 7.63 | 49.76 | 7.22 |
| SD (ng/mL) | 1.01 | 0.41 | 2.41 | 0.54 |
| CV (%) | 2.4 | 5.4 | 4.8 | 7.5 |

### ASSAY RANGE

4 – 100 ng/mL

### DETECTION LIMIT

0.5 ng/mL

### STANDARDIZATION

The calibration material used is the WHO International standard for Plasminogen Activator Inhibitor-1 (PAI-1).

## LITERATURE

*Binder BR, Geiger M. Plasminogen activator inhibitor: Biological effects. In: New Trends in Haemostasis, Coagulation Proteins, Endothelium and Tissue Factors, edited by Hasenberg, J Heene, D.L., Stehle, G., Schettler, G. Berlin, Heidelberg, New York: Springer Verlag Berlin, 1990;221-231.*