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TECHNOZYM® PAI-1 Actibind® ELISA
For research use only

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REF	TC16075	TECHNOZYM® PAI-1 Actibind® ELISA	Σ 96
REF	TC16077	TECHNOZYM® PAI-1 Actibind® Calibrator Set	5 x 0.2 mL
REF	TC16079	TECHNOZYM® PAI-1 Actibind® Control Set	2 x 0.2 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 Actibind® ELISA

GB

PRODUCT DESCRIPTION

INTENDED USE

The TECHNOZYM® PAI-1 Actibind® ELISA can be used to determine active PAI-1 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, precoated with tPA immobilized via a monoclonal anti tPA coating antibody. ELISA test strips are **not** lyophilized, but covered with adhesive film in an aluminium bag, to prevent the wells from drying.
2. Washing buffer concentrate (PBS; pH 7.3); containing detergent; 0.01% merthiolat; 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 12ml; 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 ml)
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 are made from human blood and any individual plasma involved in the procedure is HB_SAg, HIV 1/2 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, controls 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 (Greiner). 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 2500g (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 is 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 REAGENT

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. Reconstituting calibrators and control plasmas: Calibrators and control plasmas are reconstituted with **200 µl** distilled water and mixed for 10 seconds after a reconstitution time of 15 minutes (vortex mixer). Reconstituted components are clear to slightly turbid.
4. 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

WASHING (reference 1,3,4)	washing buffer	**5 x 200 µl**
SAMPLE AND CONJUGATE REACTION (reference 1,2)	Calibrators, control plasmas and samples	**25 µl**
	conjugate working solution	**75µl**
	Pipette in wells, cover test, strips with film, incubate at +37°C	**45 minutes**
WASHING (reference 1,3,4)	washing buffer	**5 x 200 µl**
SUBSTRATE REACTION (reference1,2)	pipette substrate solution into test wells	**100 µl**
	Cover test strips with film, incubate at +20 … 25°C)	**15 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

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 shall 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. Label/number strips with a water resistant pen in case the strips accidentally fall out of the frame during testing.
4. 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.
5. 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 may occur above.

ANALYSIS RESULTS

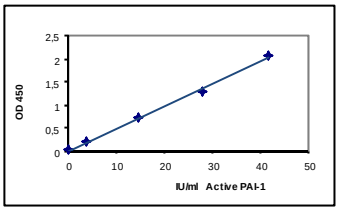
CALCULATION OF THE RESULTS

Setting up a reference curve: X axis: Concentration active PAI-1 IU/ml
Y axis: Extinction
Graph plot is linear-linear with a best fit

Assessment of reference curve

- The validity of the test may be checked on the basis of the calculated control values.

Example of standard curve.



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 (1:2 or 1:4). The measured concentration then has to be multiplied with the dilution factor 2 or 4.

REFERENCE RANGE

Normal Plasma levels range from 1-7IU/ml. It is recommended that individual laboratories establish their own normal range. Active PAI-1 levels above 20IU/ml may indicate reduced fibrinolytic capacity and, thus, increased thrombotic tendency. Measures should be taken to reduce the risk of thrombosis in individuals with elevated PAI-1 plasma levels. The TECHNOZYM® PAI-1 Actibind® ELISA exclusively measures free, active PAI-1 and is not affected by other forms of PAI-1 or other plasminogen activator inhibitors.

STANDARDISATION

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

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	10	10	3	3
Mean (IU/mL)	38.1	13.3	36.3	12.6
SD (IU/mL)	1.2	0.57	2.38	1.0
CV (%)	3.15	4.26	6.57	7.93

ASSAY RANGE

1.0 IU/mL – 85 IU/mL
(or up to the actual value of calibrator 1 and calibrator 5)

DETECTION LIMIT

0.8 IU/mL

LITERATURE

Please contact Technoclone or your local distributor.