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TECHNOZYM® t-PA Ag EDTA ELISA

For research use only

GB

REF	TC12007	TECHNOZYM® t-PA Ag EDTA ELISA	
REF	TC12001	TECHNOZYM® t-PA Antigen Calibrator Set	5 x 0.5 mL
REF	TC12003	TECHNOZYM® t-PA Antigen Control Set	2 x 0.5 mL

Symbols key			
	Manufacturer		Expiry date
	Storage temperature		Consult instructions for use
AQUA	Distilled water		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 resarch use only	WASH	Washing solution concentrate

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TECHNOZYM® t-PA Antigen EDTA ELISA

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PRODUCT DESCRIPTION

INTENDED USE

The TECHNOZYM® t-PA Antigen ELISA can be used to determine t-PA 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 t-PA 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 blue dye, 1 bottle, 90 mL, ready for use.
4. Sample dilution buffer (PBS, EDTA); contains stabiliser protein; 1 bottle, 20 mL
5. Calibrators (Standards) numbered; lyophilised; 1 bottle each. **Concentrations are lot-dependent; consult label on the vial**.
6. Control plasmas "low level" and "high level" for checking purposes lyophilised; 1 bottle each. **Concentrations are lot-dependent; consult the label on the vial.**
7. Conjugate monoclonal Anti-t-PA POX; dyed blue; 1 bottle, 0.3 mL.
8. Chromogen TMB (tetramethylbenzidine); 1 bottle, 12 mL; ready for use.
9. Stopping solution sulphuric acid 0.45 mol/L; 1 bottle 12 mL; ready for use.
10. 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 (100 mL and 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 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, 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
Sample dilution buffer concentrate	After opening	+2…8 °C	2 month
Sample dilution buffer	1+1.5 dilution of concentrate	+2…8 °C	3 weeks
Conjugate	after opening	+2...8 °C	6 months
	working solution	room temperature +18…25 °C	60 min.
Chromogen TMB	after opening	+2...8 °C	expiry date

TEST PROCEDURE

PREPARATION OF SAMPLES

Sample material: Plasma

Citrate, EDTA or CTAD 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.Thawing and refreezing of plasma aliquots is not recommended.

Haemolytic and lipaemic plasma may be used. In no case may plasma samples be used if any evidence of coagulation is seen. Venous occlusion samples are obtained by applying a tourniquet around the upper arm with the pressure between the systolic and diastolic blood pressure, e.g. 100 mm Hg, for at least 10 minutes. Draw blood from the arm before the pressure is reduced.

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). (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 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.
4. Preparing the sample dilution buffer:
   Dilute 1 part by volume sample dilution buffer with 1.5 parts by volume distilled water (e.g. 20 mL buffer + 30 mL distilled water)
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	25 µL
	Add sample dilution buffer to all wells	75 µL
	cover test strips with film, incubate at +37 °C	60 minutes
CONJUGATE REACTION (reference 1,2)	empty wells thoroughly, 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 (reference 1,2)	pipette substrate solution into test wells, cover test strip with film	100 µL
	incubate at room temperature (+18…25 °C)	20 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 for test performance

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. 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 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.

ANALYSIS RESULTS

CALCULATION OF THE RESULTS

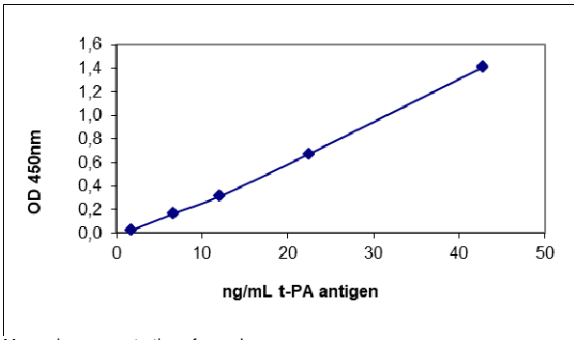
Setting up a reference curve:

X axis: Concentration t-PA antigen ng/mL
Y axis: Extinction
Graph plot is linear-linear with a linear or point to point fit

Assessment of reference curve

- The extinction coefficient of the highest calibrator should be between 1.0 and 2.5.
- The evaluability 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+1). The measured concentration then has to be multiplied with the dilution factor 2.

REFERENCE RANGE

Normal range for t-PA antigen is between 2-8 ng/mL It is recommended that individual laboratories establish their own ranges. For plasma obtained after venous occlusion values greater than 15 ng/mL, should be obtained.

A failure of t-PA levels to increase upon venous occlusion is indicative of an impaired fibrinolytic capacity and may indicate thrombotic tendencies.

STANDARDISATION

The calibration material used is the WHO international standard for tissue plasminogen activator (t-PA).

LITERATURE

I. Juhan-Vague, M.C.Alessi: Tissue-type plasminogen activator antigen. In: ECAT Assay Procedures; A manual of Laboratory Techniques; edited by J. Jespersen, R.M.Bertina and F. Haverkate, Kluwer Academic Publishers, Dordrecht, Boston, London 1992, pg 131-1