### TECHNOZYM® t-PA Ag EDTA ELISA

For research use only

<table>
<thead>
<tr>
<th>REF</th>
<th>Description</th>
<th>Catalogue number</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC12007</td>
<td>TECHNOZYM® t-PA Ag EDTA ELISA</td>
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<tr>
<td>TC12001</td>
<td>TECHNOZYM® t-PA Antigen Calibrator Set</td>
<td>5 x 0.5 mL</td>
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<tr>
<td>TC12003</td>
<td>TECHNOZYM® t-PA Antigen Control Set</td>
<td>2 x 0.5 mL</td>
<td></td>
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</tbody>
</table>

### Symbols key

- **Manufacturer**
- **Expiry date**
- **Storage temperature**
- **Consult instructions for use**
- **Distilled water**
- **Determinations**
- **Reaction buffer**
- **Lot**
- **Calibrator**
- **Microtiter plate**
- **Conjugate**
- **Catalogue number**
- **Control**
- **Ready to use**
- **Dilute or dissolve in**
- **Stop solution**
- **Incubation buffer**
- **Substrate**
- **For research use only**
- **Washing solution concentrate**

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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 and 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, 50 mL ready for use.
4. Sample dilution buffer (PBS; EDTA): contains stabiliser protein; 1 bottle, 20 mL.
5. Chloramphenicol (Stabrobesides; lyophilised; 1 bottle each. Concentrations are lot-dependent; consult the label on the vial.
6. Control plasmas "low level" and "high level" for checking purposes lyophilised; 1 bottle, 90 mL, ready for use.
7. Conjugate monoclonal Anti-t-PA POX; dyed blue; 1 bottle, 0.3 mL.
8. ELISA reader with 450 nm filter, with a 620 nm reference filter if available.

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 HBV, HIV 1/2 Ab and HCV-Ab-negative (see labels on kit and/or bottles). 

STABILITY AND STORAGE

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

Material/Reagent State Storage Stability

| Calibrators, control plasmas after reconstitution | -20 °C | 6 months |
| ELISA test strip after opening | -18...6 °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+1.15 dilution of concentrate | -2...8 °C | 3 weeks |
| Incubation buffer after opening | -2...8 °C | 2 months |
| Sample dilution buffer after opening | -2...8 °C | 2 months |
| Sample dilution buffer 1+1.15 dilution of concentrate | -2...8 °C | 3 weeks |
| Conjugate after opening | -2...8 °C | 6 months |
| Chromogen TMB after opening | -2...8 °C | expiry date |

TEST PROCEDURE

PREPARATION OF SAMPLES

Sample material: Plasma

Citrate, EDTA or CTA plasmas can be used. Centrifuge for 15 minutes at a minimum of 2500 g (DN 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 re-freezing of plasma aliquots is not recommended.

Haemolytic and lipaemic plasma may be used. In no case may plasma samples be used if any visible coagulation is seen. Venous occlusion sphygmomanometry is 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. Dilute 1 part by volume washing buffer concentrate with 51.5 parts by volume distilled water (1+11.5). (Diluted washing buffer concentrate = washing buffer).

2. Prepare 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 crystals or 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

SUBSTRATE REACTION (reference 1,2)

Empty wells thoroughly, pipette conjugate working solution into wells cover test strip with film

100 µL

WASHING (reference 1,3,4)

Incubate at +37 °C

60 minutes

STOPPING (reference 1,2)

Incubate at room temperature (+18...+25 °C)

20 minutes

MEASURING (reference 5)

ElizA-Reader, 450 nm

Shake 10 sec., measure within 10 minutes

References for test performance:

1. Reagents of different lots might 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 ± 15 %.
   - 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 (6 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.
   - Label/number strips with a water resistant pen in case the strips accidentally fall out of the frame during testing.
   - 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.
   - 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

PERFORMANCE OF THE TEST

Sample(s) which fall higher than the top calibrator standard must be retested at a higher dilution as a "hook dose" response may occur. For samples which fall higher than the top calibrator standard, the time for sample incubation, conjugate and substrate reaction must be increased.

LIMITATION OF THE TEST

The extinction coefficient of the highest calibrator should be between 1.0 and 2.5.

REFERENCE RANGE

Normal range for t-PA antigen is between 2-8 ng/mL. It is recommended that individual laboratories establish their own ranges.

STANDARDISATION

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

LITERATURE