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**STANDARD OPERATING PROCEDURE for tissue Plasminogen Activator  
(tPA) antigen ELISA**

(Biopool, Umea Sweden. See kit insert)

Principle of assay

This assay employs a double antibody sandwich ELISA.

Reagents

Coating antibody:

96 well microtitre plates are pre-coated with goat anti-human tPA IgG.  
Plasma sample or standard containing tPA is added to a micotest well. An incubation period allows 95% of tPA to bind to the coating antibody.

Detection antibody:

HRP-labelled Fab fragments of anti tPA IgG are added to each well and incubated to allow reaction with the bound tPA. The wells are emptied and washed to remove unbound conjugate. 1,2 phenylenediamine dihydrochloride substrate (OPD) and hydrogen peroxide (as activator) are then added, and the released yellow product is measured photometrically on a microplate reader. The colour development is proportional to the concentration of tPA in the sample. After stopping the reaction with H<sub>2</sub>SO<sub>4</sub>, the concentration is read from the standard curve on the same plate.

Preparation of Reagents and materials in the kit

96 well microtitre plates precoated with goat anti-human tPA IgG.

Remove from

*Standard 1:* 0 ng/ml standard containing tPA- depleted lyophilised human plasma.

*Standard 2:* 30 ng/ml standard containing lyophilised human plasma enriched with human single-chain tPA which has been calibrated against the international standard for tPA (NIBSC)

These standards contain merthiolate as a preservative.

*Conjugate*

Lyophilised HRP-labelled anti-human tPA Fab fragments.

Prepared by adding 7 ml of bu

*PET buffer*

PBS-EDTA-Tween 20 buffer substances sufficient to make a 1litre solution. Dissolve in 1 litre of water and mix with a magnetic stirrer for about 15 minutes.

*Substrate*

Lyophilised 1,2 phenylediamine dihydrochloride (OPD) in buffer salts.

*Hydrogen peroxide*

2 ml 0.15% H<sub>2</sub>O<sub>2</sub> in water.

## **Other reagents and materials.**

### **Controls:**

An in-house control of pooled plasma is used as a control on each plate  
The tPA mean concentration of this plasma is assigned after at least 20 separate runs.  
A Levy-Jennings curve is generated, and QC results are plotted on this for each run.  
Westgard rules are used to monitor internal quality control.

### **Acid (Stop) Solution**

Slowly add 50ml of concentrated sulphuric acid to 540ml water in fume cupboard.  
This solution will be quite hot. Cover, label and allow to cool.

GLOVES MUST BE WORN WHEN HANDLING OPD (carcinogenic)  
HYDROGEN PEROXIDE AND ACID (corrosive).

### **Preparation of the standard curve.**

Add 0.5 ml distilled water to each of the two standards and gently agitate for 5 minutes to completely dissolve contents. Prepare the curve as follows:

Concentration (ng/ml)	30 ng/ml Standard ( $\mu$ l)	0 ng/ml Standard ( $\mu$ l)
0	0	200
10	75	150
20	150	75
30	200	0

### **Wash Procedure**

The plate should be washed between incubations as follows:  
Fill each well with washing buffer.  
Soak for 20 seconds.  
Invert the plate and decant the buffer into sink.  
Remove excess buffer by grasping the plate firmly and smartly rapping the inverted.  
plate onto a clean paper towel.  
This procedure should be performed 3 times.  
Alternatively the MRW plate washer may be use using a suitable programme.

### **Assay procedure**

Make up worksheet using a 96 well template format and enter standard and sample details.  
Add 100 $\mu$ l of diluted standard, sample and control to the appropriate wells.  
Incubate at RT for 30mins on plate shaker at a setting of 5.

Wash X3

Add 100 µl conjugate (HRP) prepared as above.  
Incubate at RT for 30mins on plate shaker as before.

Wash X3

Add 100 µl substrate prepared as above. Substrate should be added to each row at 5 or 10 second intervals.

Incubate at RT for 3mins on bench.

Add 100 µl 1M H<sub>2</sub>SO<sub>4</sub> (stop). The acid should be added to the rows at the same intervals (5 or 10 seconds) as the addition of the substrate to ensure that each well has the same incubation time.

Read immediately on plate reader MR5000 at 492nm using the relevant programme. This will plot a linear curve and read the sample ODs from it. Plates should be read as soon as possible because colour development may continue after the addition of the acid.

Write results onto the work sheet and then key into the relevant data-base.  
Any samples with values below 0.5 ng/ml should be repeated. Samples with values above 30 ng/ml should be diluted 1 in 2 with 0 ng/ml standard and re-run. File worksheets in correct study folder. The control result is plotted onto the Levey-Jennings chart. Westgard quality assurance rules must be observed.  
Discard the contents of the plate down the sink and flush through with plenty of cold running water.