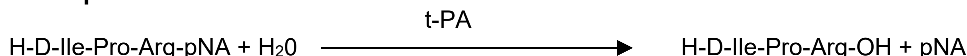
# Tissue Plasminogen Activator (t-PA)

## Method Sheet S-2288

### Determination of Tissue Plasminogen Activator (t-PA) in Purified Preparations

### Principle:




H-D-Ile-Pro-Arg-pNA + $H_2O$ $\xrightarrow{\text{t-PA}}$ H-D-Ile-Pro-Arg-OH + pNA

The tissue plasminogen activator (t-PA) is a family of serine proteases which activate plasminogen by splitting a single Arg-Val bond of the plasminogen molecule. In purified systems these enzymes have been shown to hydrolyse tripeptide chromogenic substrates (1, 2, 3).
The t-PA activity is thus determined by the rate at which p-nitroaniline (pNA) is released. The formation of pNA can be followed spectrophotometrically at 405 nm by using a recorder (initial rate method).
The correlation between the change in absorbance per minute (ΔA/min) and the t-PA acitivity is linear in the 0.05 - 0.5 µkat/l or 3 - 30 U/l range.
The amidolytic activity does not necessarily parallel the fibrinolytic activity for different t-PA preparations.

### Reagents:

**1. Substrate S-2288 (H-D-Ile-Pro-Arg-pNA • 2HCl) mol.wt. 577.6.**
The substance (25 mg) is dissolved in 8.65 ml (t-PA one-chain) or 43 ml (t-PA two-chain) of distilled water. If prepared with sterile water and not contaminated by microorganisms, the 5 mmol/l or 1 mmol/l solution is stable for at least two months at 2 to 8°C.

**2. Buffer pH 8.4 (25°C)**

| | |
|---|---|
| Tris | 12.1 g (100 mmol/l) |
| NaCl | 6.2g (106 mmol/l) |
| Distilled water | 800 ml |

Adjust the pH to 8.4 at 25°C by adding an appropriate amount (approximately 44 ml) of 1 mol/l HCl. Fill up to 1000 ml with distilled water. The buffer, if not contaminated, is stable for six months at 2 to 8°C.

**3. Acetic acid 20%**

Acetic acid is used in the acid-stopped method.

Equipment :
1. Spectro- or filter photometer, 405 nm with cuvette housing, thermostated at 37°C
2. Semi-microcuvettes, 1 cm
3. Thermostat, 37°C
4. Stop watch
5. Disposable plastic tubes

### Procedure:

**Sample:**
Purified tissue plasminogen activator is dissolved in buffer to an enzyme acitivity of 0.05 - 0.5 µkat/l (3 - 30 U/l). See note*.
It has been advised to use a surfactant to avoid adsorption to surfaces. A final concentration of 0.1 g/l of Triton X-100 (Merck) is recommended.

**Method:**

| Initial rate method | | Volume |
|---|---|---|
| Buffer | µl | 200 |
| Thermostate at 37°C (2-4 min) | | X |
| Sample (20-25°C) | µl | 200 |
| Mix and thermostate at 37°C (2-4 min) | | X |
| S-2288 (37°C) | µl | 200 |
| Mix | | X |

Transfer sample immediately to a 1 cm semi-microcuvette (preheated to 37°C) for measurement of the absorbance change in a photometer at 405 nm and at 37°C. Calculate ∆A/min.

**Calculation:**
The t-PA activity in the prepared tissue plasminogen activator solution is calculated from the following formulas:
µkat/l = ∆A/min x 5.21
U/l = ∆A/min x 313

***Note:**
In the test (600 µl) 0.25 µg (≅ 100 IU) of the procine heart tissue activator gives:
∆A/min ≅ 0.012 (one-chain)
∆A/min ≅ 0.065 (two-chain)

**Bibliography**

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