

## Tissue Plasminogen Activator (t-PA)

Determination of t-PA in purified preparations with S-2288

### Measurement Principle

Tissue plasminogen activator (t-PA) is a serine protease, which activates 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. 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 (DA/min) and the t-PA activity is linear in the 0.05 - 0.5  $\mu\text{kat/l}$  or 3 - 30 U/l range. The amidolytic activity does not necessarily parallel the fibrinolytic activity for different t-PA preparations.



### Reagents

1. S-2288, 25 mg Art. No. 82 08 52  
Reconstitute the substrate S-2288 (MW: 577.6) with 8.65 ml (t-PA one-chain) or 43 ml (t-PA two-chain) of distilled water.
2. Tris Buffer, pH 8.4 (25°C)

Tris	12.1 g	(100 mmol/l)
NaCl	6.2 g	(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 2-8°C.

3. Acetic acid 20%  
Acetic acid is used in the acetic-stopped method.

### Sample

Purified tissue plasminogen activator is dissolved in buffer to an enzyme activity of 0.05 - 0.5  $\mu\text{kat/l}$  (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 is recommended.

### Method

Initial rate method	
Buffer	200 $\mu\text{l}$
Incubate at 37°C	2-4 min
Diluted sample (20-25°C)	200 $\mu\text{l}$
Incubate at 37°C	2-4 min
Substrate (37°C)	200 $\mu\text{l}$
Mix	

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  $\Delta A/\text{min}$ .

### Calculation

The t-PA activity in the prepared tissue plasminogen activator solution is calculated from the following formulas:

$$\mu\text{kat/l} = \Delta A/\text{min} \times 5.21$$

$$\text{U/l} = \Delta A/\text{min} \times 313$$

Note:

*In the test (600  $\mu\text{l}$ ) 0.25  $\mu\text{g}$  (100 IU) of the porcine heart tissue plasminogen activator gives:*

*$\Delta A/\text{min} \sim 0.012$  (one-chain)*

*$\Delta A/\text{min} \sim 0.065$  (two-chain)*

### Bibliography

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