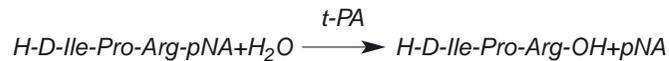


TISSUE PLASMINOGEN ACTIVATOR (t-PA)

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 ($\Delta A/\text{min}$) 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. S820852
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 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

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 μl
Incubate at 37°C	2-4 min
Sample (20-25°C)	200 μl
Mix and incubate at 37°C	2-4 min
Substrate (37°C)	200 μl
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:

$$\begin{aligned} \mu\text{kat/l} &= \Delta A/\text{min} \times 5.21 \\ \text{U/l} &= \Delta A/\text{min} \times 313 \end{aligned}$$

Note: In the test (600 μl) 0.25 μg (100 IU) of the porcine heart tissue plasminogen activator gives:
 $\Delta A/\text{min} \cong 0.012$ (one-chain)
 $\Delta A/\text{min} \cong 0.065$ (two-chain)

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