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 proteases, 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 (ΔA/min) and the t-PA activity 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.

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

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 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 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 ΔA/min.

Calculation

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

Note: In the test (600 µl) 0.25 µg (100 IU) of the porcine heart tissue plasminogen activator gives:
ΔA/min ≅ 0.012 (one-chain)
ΔA/min ≅ 0.065 (two-chain)

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