PLASMIN ACTIVITY

Determination of plasmin activity with S-2251.

Principle: Plasmin hydrolyses the chromogenic substrate S-2251 and liberates the chromophoric group pNA. The color is then read photometrically at 405 nm.

Reagents: S-2251, 25 mg: Reconstitute with 13.0 ml sterile water (3.5 mM)
Albumin, human: 20%
Buffer stock solution: Tris 0.5 M, pH 7.4, 10 ml.
Dilute 1 + 9 with sterile water to obtain working solution (1).
Mix working solution (1), 100 ml + human albumin, 2500 µl to obtain working solution (2).
Mix working solution (2), 17.0 ml + substrate S-2251, 5.0 ml

Sample: Dilute sample with working solution (2) to a suitable level of approximately 2 – 5 nkat/ml.

Method:

Working solution (2) + substrate (37°C)	900 µl
Plasmin (20 - 25°C)	100 µl
Mix and read directly at 405 nm	

Read, for 2 minutes ΔA_{405}/min. at 37°C, according to instructions for the calibrated instrument, using water for zero adjustment. Run a duplicate for each sample in a plastic cuvette preheated to 37°C.

Note: It is of crucial importance that the wavelength of the photometer is 405 nm. This should be checked by a suitable pNA solution or equivalent photometer calibration device.

Calculations: ΔA_{405}/min x dilution x factor = nkat/ml

$$\text{Factor:} \quad \frac{V}{\theta \times t \times 10^{-9} \times 10^{3} \times v}$$

$\theta = 9650\ mol^{-1}L$
v = 0.1 mL = sample volume
V = 1.0 ml = total volume in cuvette
t = 60 s = time

Sample: ΔA_{405}/min x dilution x 17.27 = nkat/ml