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.

**Calculations:**

\[
\Delta A_{405/min} \times \text{dilution} \times \text{factor} = \text{nkat/ml}
\]

**Factor:**

\[
\frac{\theta \times t \times 10^{-9} \times 10^3 \times V}{0.1 \text{ mL} \times \text{sample volume}}
\]

\[
\theta = 9650 \text{ mol}^{-1}\text{L}
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\[
v = 0.1 \text{ mL} = \text{sample volume}
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\[
V = 1.0 \text{ mL} = \text{total volume in cuvette}
\]

\[
t = 60 \text{ s} = \text{time}
\]

**Sample:**

\[
\Delta A_{405/min} \times 17.27 = \text{nkat/ml}
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**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.