URINE KALLIKREIN

Determination of kallikrein in urine with S-2266

Measurement Principle

Kallikrein in urine hydrolyses the substrate H-D-Val-Leu-Arg-pNA (S-2266) and the rate of p-nitroaniline (pNA) formation increases linearly with increasing concentration of kallikrein up to 30 nkat/l. (See note). By adding aprotinin, a potent inhibitor of glandular kallikrein, to the sample blank, protease activities not inhibited by aprotinin as well as the colour from the urine itself can be subtracted.

$$\text{H-D-Val-Leu-Arg-pNA} + H_2O \xrightarrow[\text{kallikrein}]{\text{urinary}} \text{H-D-Val-Leu-Arg-OH} + \text{pNA}$$

Reagents

1. S-2266, 25 mg Art. No. S820480
Reconstitute the substrate S-2266 (MW: 579.6) with 28.8 ml of distilled water.

2. Tris Buffer, pH 8.2 (25°C)

Tris	24.4 g	(200 mmol/l)
Distilled water	800 ml	

Adjust the pH to 8.2 at 25°C by adding an appropriate amount of 1 mol/l HCl (approximately 100 ml with distilled water. Fill up to 1000 ml with distilled water. The buffer, if not contaminated, is stable for two months at 2 -8°C.

3. Trasylol® buffer
Trasylol (lyophilized aprotinin) is added to the buffer (Reagent 2) to a concentration of 20 KIU/ml.

4. Acetic acid, 50%

Equipment

1. Spectro- or filter photometer, 405 nm
2. Semi-microcuvettes, 1 cm
3. Centrifuge
4. Thermostat, 37°C
5. Stop-watch
6. Disposable plastic tubes

Specimen collection

As the kallikren concentration may vary during the day, the total volume collected during 24 hours should be pooled. No drugs should be taken on the day of the sampling unless it is the aim to evaluate the influence of the drug in the kallikrein secretion. After mixing the urine pool a portion is transferred into a disposable plastic tube and kept at 2-8°C (less than 24 hours) or below -20°C. Just before the analysis, the urine sample is centrifuged and the supernatant is used.

Method

Acid stopped method	Sample	Blank
Buffer	500 µl	-
Trasylol	-	500 µl
Incubate at 37°C	5-10 min	5-10 min
Urine	400 µl	400 µl
Mix and incubate at 37°C	2-5 min	2-5 min
Substrate (37°C)	100 µl	100 µl
Mix and incubate at 37°C	30 min	30 min
Acetic acid 50%	100 µl	100 µl

Read the absorbance (A) of the sample against its blank in a photometer at 405 nm. The colour is stable for at least 4 hours.

Calculation

The activity of kallikrein per litre of urine or excrete during 24 hours is calculated from the formula:

nkat/l	= 159 x A	U/l	= 9.55 x A
nkat/24hr	= 159 x v x A	U/24hrs	= 9.55 x v x A

A = absorbance
v = total volume in litre of urine collected during 24 hours.

Note: If the kallikrein activity exceeds 30 nkat/l the urine should be diluted with the same volume of buffer and the result multiplied with 2.

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