PLASMA KALLIKREIN-LIKE ACTIVITY

Determination of kallikrein-like activity in plasma, with S-2302.

Measurement Principle

The plasma kallikrein-like activity catalyses the splitting of p-nitroaniline (pNA) from the substrate H-D-Pro-Phe-Arg-pNA (S-2302). The rate at which the pNA is released is measured photometrically at 405 nm. This can conveniently be read after stopping the reaction with acetic acid (acid stopped method). The activity measured is mainly the kallikrein-α2-macroglobulin complex.

Kallikrein-like activity

H-D-Pro-Phe-Arg-pNA + H_2O ⟶ H-D-Pro-Phe-Arg-OH + pNA

Reagents

1. S-2302, 25 mg Art. No. S820340
Reconstitute the substrate S-2302 (MW: 611.6) with 20 ml of distilled water.

2. Tris Buffer, pH 7.8 (25°C)

Tris	6.1 g	(50 mmol/l)
NaCl	6.6 g	(113 mmol/l)
Distilled Water	800 ml	

Adjust the pH to 7.8 at 25°C by adding an appropriate amount (approx. 38 ml) of 1 mol/l HCl. Fill up to 1000 ml with distilled water. The buffer, if not contaminated, will remain stable for six months at 2 to 8°C.

3. Acetic acid, 20%

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

Blood (9 vol) is mixed with 0.1 mol/l sodium citrate (1 vol) and centrifuged at 2000 x g for 20 minutes at 15-25°C. In order to avoid low-temperature activation of prekallikrein the plasma should be kept at 15-25°C for not more than a few hours or immediately frozen at -20°C or below. After thawing at 37°C the plasma should be kept at 15 to 25°C and used as soon as possible. Frozen plasma may loose some kallikrein-like activity on freezing or thawing, but is stable for several months at -20°C or below.

Method

Sample dilution	Tube No. 1
Buffer	1000 µl
Test plasma	100 µl
Mix	

Acid stopped method	Tube No. 2
Sample from tube No. 1	200 µl
Incubate at 37°C	3-4 min
Substrate	200 µl
Mix and incubate at 37°C	10 min
Acetic acid, 20%	200 µl
Mix	

Plasma blanks are prepared by adding the reagents in reverse order without incubation. 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

Plasma kallikrein-like activity in enzyme activity units:
µkat/l = A x 5.73
U/l = A x 344

Notes
1. The substrate S-2302 is also sensitive to plasmin. By testing with 2 mmol/l S-2251 it is possible to check whether plasmin is present in the sample.
The substrate S-2251 is not sensitive to kallikrein.
2. If the method is to be used for subtraction of blank activities in the prekallikrein assay, it may be preferable to dilute the plasma as indicated for that assay.

Bibliography

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