KALLIKREIN INHIBITOR ACTIVITY

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

Measurement Principle

Plasma is incubated with a purified plasma kallikrein preparation. The amount of kallikrein inhibited is proportional to the activity of the kallikrein inhibitor present in the plasma. The remaining amount of kallikrein activity is then determined by using the substrate H-D-Pro-Phe-Arg-pNA (S-2302).
The rate at which p-nitroaniline (pNA) is released is measured photometrically at 405 nm. This can be followed on a recorder (initial rate method) or read after stopping the reaction with acetic acid (acid stopped method).

K-Inhibitor + K (excess) ⟶ *[K-Inhibitor•K] + K (residual)*

K (residual)
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. Plasma Kallikrein
Use purified human plasma kallikrein (refer to Gallimore MJ et al., 1978). Prepare a solution of 1 $nkat_{S\text{-}2302}$/ml human plasma kallikrein in Tris buffer pH 7.8.
1 $nkat_{S\text{-}2302}$ corresponds to 0.06 U or 0.017 PEU (refer to Friberger P et al. 1979).

3. Tris Buffer, pH 7.8 (25°C)

Tris	6.1 g	(50 mmol/l)
NaCl	21.1 g	(361 mmol/l)
Polybrene	20 mg	
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.

4. Normal plasma
Blood samples are taken from at least 10 healthy donors. For the preparation of the samples, refer to the Specimen collection section.

5. Acetic acid, 20%
Acetic acid is used in the acid stopped method.

Equipment

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

Additional equipment for the initial rate method
7. Photometer with cuvette housing, thermostated at 37°C.

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 plasma kallikrein inhibitor 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 15-25°C and used as soon as possible. Frozen plasma may lose some plasma kallikrein inhibitor activity on freezing or thawing, but is stable for several months at -20°C or below.

Standard curve

Normal plasma has a kallikrein inhibitor concentration of 100% and is diluted according to the table below (see Note 1).

K-Inhibitor %	Normal plasma µl	Buffer µl
25	100	300
50	200	200
75	300	100
100	400	-

Method

Sample dilution	Tube No. 1
Buffer	1900 µl
Test plasma or standard (see note 1)	100 µl
Mix	

Initial rate method	Tube No. 2
Sample from tube No. 1	200 µl
Incubate at 37°C	3-4 min
Plasma kallikrein	200 µl
Mix and incubate at 37°C	5 min
Substrate (37°C)	200 µl
Mix	

Transfer sample immediately to a 1 cm siliconised 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.

Acid stopped method	Tube No. 2
Sample from tube No. 1	200 µl
Incubate at 37°C	3-4 min
Plasma kallikrein	200 µl
Mix and incubate at 37°C	5 min
Substrate (37°C)	200 µl
Mix and incubate at 37°C	4 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 inhibitor in percentage of normal plasma
Plot A or ΔA/min for the standards against their concentration of kallikrein inhibitor on log-lin graph paper.
Read the kallikrein inhibitor value for the corresponding A or ΔA/min of the unknown test sample from the standard curve.

Plasma Kallikrein inhibitor in enzyme activity units
In each test series a kallikrein activity determination with buffer instead of sample dilution must be performed.
The difference between this activity and the sample activity is then calculated.
Initial rate method:

μkat/l = (ΔA/min buffer - ΔA/min sample) x 104
U/l = (ΔA/min buffer - ΔA/min sample) x 6 250

Acid stopped method:

μkat/l = (Abuffer-Asample) x 34.7
U/l = (Abuffer-Asample) x 2 080

Notes
1. A 150% standard is prepared by diluting 300 µl normal plasma with 3700 µl buffer. A 200% standard is prepared by diluting 100 µl normal plasma with 900 µl buffer. For 0% use the buffer only (note that the adsorbance to surfaces can result in lower readings when plasma is absent).
2. It is suggested, that the spontaneous kallikrein activity (α2-M complex) should be determined in patients in whom the kallikrein system is suspected to be activated.
See Determination of Kallikrein-like Activity in Plasma.

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