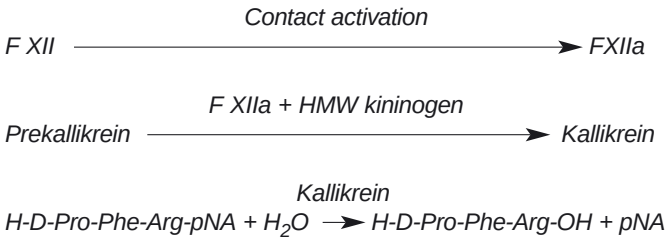
PLASMA PREKALLIKREIN

Determination of prekallikrein in plasma with S-2302.

Measurement Principle

The activation of plasma prekallikrein is mediated by the Hageman factor on negatively charged surfaces and in presence of HMW kininogen. A number of methods have been described for the activation of prekallikrein.
This method is related to the use of a prekallikrein activator composed by ellagic acid, phospholipid, Hageman factor and HMW kininogen, which was commercially available from Chromogenix. Different batches of this prekallikrein activator, not available any more, were assayed against the first PKA international standard and resulted in an activity of about 100-120 IU per vial. In the “Reagents” section the main characteristics of the prekallikrein activators have been reported. However, the assay should be validated with respect to the particular activator used.
Following the activation, the plasma kallikrein formed catalyses the hydrolysis of p-nitroaniline (pNA) from the substrate H-D-Pro-Phe-Arg-pNA (S-2302). The rate at which 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).
The concentration of prekallikrein is calculated by using standards prepared from normal plasma.



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 Prekallikrein Activator
Prepare a solution of plasma prekallikrein activator (PKA) with an activity of 20-24 IU/ml. This component should contain ellagic acid, phospholipid and a plasma fraction composed by Hageman factor and high molecular weight kininogen. (Meier H et al. 1977; Wiggins RC et al. 1977; Friberger P et al. 1979; Kerry PJ et al. 1985).
In alternative refer to Claeson G et al. 1978; Friberger P et al. 1979; Shibuya Y et al. 1991, to use other prekallikrein activators.

3. Tris Buffer, pH 7.8 (25°C)

Tris	6.1 g	(50 mmol/l)
Distilled water	800 ml	

Adjust the pH to 7.8 at 25°C by adding an appropriate amount (approximately 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 2-8°C.

4. Normal plasma
Blood should be taken from at least 20 healthy donors. 10-30 ml of citrated blood (9 vol blood and 1 vol 0.1 mol/l sodium citrate) are taken from each donor (the first ml of blood is discarded). Plasma is prepared by centrifugation at 2000 x g for 20 minutes at 15-25°C. Equal amounts of plasma from the donors are mixed and dispensed in small volumes. In order to avoid low-temperature activation of prekallikrein the plasma should be kept at 15-25°C and used as soon as possible or quickly 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 is stable for three months at -20°C or below. Avoid refreezing.

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

Equipment

1. Spectrophotometer or microplate reader
2. Semimicrocuvettes, 1 cm or microplates
3. Centrifuge
4. Thermostat, 37°C
5. Stop watch
6. Disposable plastic tubes
8. Photometer with cuvette housing, thermostated at 37°C (for the initial rate method)

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 plasma should be kept at 15-25°C for not more than 24 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 loose some prekallikrein on freezing or thawing, but will remain stable for three months at -20°C or below. Avoid refreezing.

Standard curve

The normal plasma has a prekallikrein concentration of 100% and is diluted according to the table below.

Prekallikrein %	Normal plasma µl	Buffer µl
25	100	300
50	200	200
75	300	100
100	-	-
125	see below	-

Method

Sample dilution	Tube No. 1
Buffer	3000 µl
Test plasma or standard	50 µl
Mix	

To obtain the 125% standard, mix 125 µl normal plasma with 6 ml buffer.
The test tube method or the Microplate method can be performed by the acid-stopped or the initial rate methods.

Acid stopped method	Test tube	Microplate
Prekall. Activator	200 µl	50 µl
Incubate at 37°C	3-4 min	3-4 min
Sample from tube No. 1	200 µl	50 µl
Mix and incubate at 37°C	2 min*	2 min*
Substrate (37°C)	200 µl	50 µl
Mix and incubate at 37°C or read the initial rate	2 min	2 min
Acetic acid 20%	200 µl	50 µl

**The incubation time depends from the prekallikrein activator used. 2 min is the incubation time with a PKA with the characteristics described in the reagent section.*

For the acid-stopped method: read the absorbance at 405 nm within 4 hours. If the plasma is icteric, hemolytic or lipemic, plasma blanks should be determined. Plasma blank is prepared by adding the reagents in reverse order starting with the acetic acid, without incubation. Subtract the absorbance of the blank from the absorbance of the corresponding sample. For the initial rate method in test tubes: transfer sample immediately after addition of the substrate to a 1 cm semi-microcuvette (preheated at 37°C) for measurement of the absorbance change at 405 nm.

Calculation

Plot A or ΔA/min for the standards against their concentration of prekallikrein on linear graph paper. Read the prekallikrein value for the corresponding A or ΔA/min for the unknown test sample from the standard curve.

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