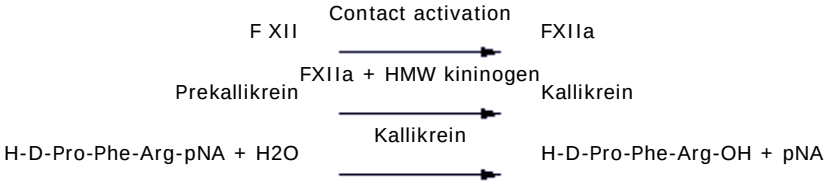
Plasma Prekallikrein

Determination of prekallikrein in plasma with a chromogenic substrate

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 human FXIIa (Hageman factor) acting as prekallikrein activator. 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 chromogenic substrate H-D-Pro-Phe-Arg-pNA (CS-31(02)). 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. Chromogenic substrate e.g. CS-31(02), 25 mg; art.no.: 229031
   Reconstitute with 20 mL of distilled water.
2. Human Factor XIIa - Prekallikrein Activator, 100 ng; art.no.: EZ012A
   Reconstitute with 5 mL of Tris-buffer (3).
3. Tris-Buffer pH 7.8; art.no.: AR103A (10 mL)
4. Calibration Plasma; art.no.: CCNRP-05 (25x0.5 mL), CCNRP-10 (25x1.0 mL)
5. Acetic acid, 20% or citric acid 2%, used in the acid-stopped method only.

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.

Calibration curve

The calibration plasma has a prekallikrein concentration of e.g. 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	
Buffer	3000 µL
Test plasma or calibrator/control	50 µL

To obtain the 125% calibrator, mix 125 mL 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 method.

	Test tube method	Microplate method
Hageman factor - Prekallikrein activator#	200 µL	50 µL
Incubate at 37°C	3-4 min	3-4 min
Diluted sample/calibrator/control	200 µL	50 µL
Incubate at 37°C	2 min*	2 min*
Substrate (37°C)	200 µL	50 µL
Incubate at 37°C or read the initial rate	2 min	2 min
Acetic acid 20% or citric acid 2%	200 µL	50 µL

*The incubation time depend on the prekallikrein activator used.
lot specific, pre-testing might be required

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 calibration curve.

Bibliography

1. Claeson G et al. Methods for determination of prekallikrein in plasma, glandular kallikrein and urokinase. Haemostasis 7, 76-78 (1978).
2. Friberger P et al. Determination of prekallikrein in plasma by means of a chromogenic tripeptide substrate for plasma kallikrein. In: KININS II. Biochemistry, Pathophysiology and Clinical Aspects. Eds. Fujii S, Moriya H & Suzuki T. Plenum Publishing Corp, 67-82 (1979).
3. Sakuragawa N et al. Changes of prekallikrein in the cases with disseminated intravascular coagulation syndrome. In KININS II. Systemic Proteases and Cellular Function. Eds Fujii S, Moriya H & Suzuki T. Plenum Publishing Corp, 185-193, (1979).
4. Aasen AO et al. Studies on components of the plasma kallikrein-kinin system in plasma samples from normal individuals and patients with septic shock. Advances in Shock Research 4. Ed A. Lener. Alan R. Liss Inc. 1-10 (1980).
5. Gallimore MJ et al. Further studies on components of the plasma kallikrein system in plasma samples from cancer patients and normal individuals. In Progress in Chemical Fibrinolysis and Thrombolysis. Vol. V. Ed Davidson J F, 256-258 (1980).
6. Gallimore MJ et al: Simple chromogenic peptide substrate assays for determining prekallikrein, kallikrein inhibition and kallikrein "like" activity in human plasma. Thromb Res 25, 293-298 (1982).
7. Friberger P et al. Chromogenic substrates for kallikreins and related enzymes. Agents and Actions. Suppl 9, 83-90 (1982).
8. Kerry PJ et al. Standardization of prekallikrein activator (PKA): the 1st International Standard for PKA. Br J Haematol 60, 345-352 (1985).
9. Friberger P and Gallimore MJ. Description and evaluation of a new chromogenic substrate assay kit for the determination of prekallikrein in human plasma. In KININS IV, 543-547 (1986).
10. De La Cadena R et al. Evaluation of a microassay for human plasma prekallikrein. J Lab Clin Med 109, 601-607 (1987).
11. Gallimore MJ and Friberger P. Prekallikrein activator, Letter to the Editor. Thromb Haemost 52, 366 (1984).
12. Shibuya Y et al. Mechanized assay of plasma prekallikrein by activation with Pseudomonas aeruginosa elastase and amidolysis of chromogenic substrate. Clin Chim Acta 200, 119-128 (1991).

CoaChrom Diagnostica GmbH
Hauptstrasse 5, 2344 Maria Enzersdorf, Austria
info@coachrom.com www.coachrom.com
T: +43-1-236 222 1 F: +43-1-236 222 111
Toll free from Germany:
T: 0800 - 246 633 0 F: 0800 - 246 633 3