CoaChrom® KKI

Chromogenic Assay for Inhibitors of Plama Kallikrein in Human Plasma

Art.No. COA0073

For Research Use Only

This kit is designed for the determination of inhibitors of Plasma Kallikrein in human plasma. Plasma Kallikrein is added to diluted plasma and during an incubation period it complexes with plasma inhibitors. The residual Plasma Kallikrein activity is measured by its ability to cleave a chromogenic peptide substrate and liberate p-nitroaniline (pNA). The concentration of pNA is measured photometrically, and is inversely proportional to the plasma inhibition of Kallikrein[1].

REAGENTS

The reagents should be stored at 2-8°C until reconstituted.

1. Chromogenic Kallikrein Substrate, 10 ml 1 vial

10 µmol/vial MBz-Pro-Phe-Arg-pNA plus mannitol. Reconstitute with 10 ml aqua dest. Stable for at least 6 months at 2-8°C.

2. Human Plasma Kallikrein, 10 ml 1 vial

Lyophilised preparation containing approximately 0.1 PEU (Plasma Equivalent Units) or 30-50 µg of Plasma Kallikrein, stabilisers and buffer salts. Reconstitute with 10 ml aqua dest. Stable for 6 hours at 2-8°C or 6 months at -80°C if it is snap frozen immediately after reconstitution, although a small loss of activity may be found on freezing and thawing.

3. Buffer Concentrate, 10 ml 2 vials

Dilute the buffer concentrate 1+9 with distilled water. This gives an assay buffer of 0.05 M Tris-HCl, 0.15 M NaCl, pH 7.8. Store at 2-8°C.
Diluted buffer should be used within 24 hours.

4. Standard Plasma, 1 ml 1 vial

Add 1.0 ml aqua dest, leave for 5 minutes at room temperature and then mix gently until completely dissolved. The potency value of the standard is printed on the vial label. Stable for 8 hours at 2-8°C or 6 months at -20°C.

Material required, but not provided: 20 % Acetic acid or 2 % Citric acid, 10 ml plastic tube for chromogenic Substrate dilution.

BLOOD COLLECTION AND PLASMA PREPARATION

Blood (9 parts) is mixed with 0.106 M Tri-sodium citrate (1 part) and centrifuged at 2000 g for 15 minutes at room temperature. The plasma samples should be removed with plastic pipettes within two hours of blood collection and should be assayed immediately or stored frozen at -20°C.

PREPARATION OF THE STANDARD CURVE

Dilute the Standard Plasma (Reagent 4) with assay buffer as follows:

Standard (%)	Plasma (µl)	Buffer (µl)
150	150	850
100	100	900

From the 100 % Standard, prepare:

75	300	100
50	200	200
25	100	300
0	Use assay buffer alone	

SAMPLE PRE-DILUTION

Dilute 100 µl of each test plasma with 900 µl of assay buffer.

ASSAY METHOD

Have the Substrate at 37°C and keep the plasma dilutions at room temperature. Into siliconised semi-micro cuvettes, siliconised glass or plastic tubes pipette:

Plasma dilution or buffer 200 µl

Incubate at 37°C for 2 minutes, add:

Plasma Kallikrein (Reagent 2) 200 µl

Mix and incubate at 37°C for 5 minutes, add:

Chrom. Kallikrein Substrate (Reagent 1) 200 µl

Mix and record the change in optical density per minute at 405 nm (rate assay), or incubate for exactly 15 minutes at 37°C, add:

Acetic acid (20 %) or Citric acid (2 %) 200 µl

Mix and read OD at 405nm (end point assay).

Microtitre Method
Follow the manual method above, but pipette 50 µl volumes of each plasma dilution and reagent into the wells of a microtitre plate. Care must be taken to ensure adequate mixing after each reagent addition.

CALCULATION
For the end point assay, prepare blanks by substituting 400 µl assay buffer for the Plasma Kallikrein and Chromogenic Substrate (microplate method: 100 µl). Subtract the blank values from the test values. Plot the results as log A_{405} against percentage Kallikrein Inhibition for the standard plasma dilutions and read the values for the test plasmas from the standard curve.
The final results can be expressed either as a percentage of standard, or in units per ml (U/ml) by applying the formula:

Plasma Kallikrein Inhibitors (U/ml) =

$$\frac{\% \text{ Activity x Potency of Standard}^{*}}{100}$$

*The potency value of the Standard Plasma is printed on the vial label.

PERFORMANCE CHARACTERISTICS
The assay is linear up to 150 %, with a sensitivity limit of 5 %. The intra-assay coefficient of variation is 5 % at 1.00 U/ml.

INTERPRETATION
Normal Range: A 2 SD range of 0.85 - 1.29 U/ml (85-129 %) was obtained using citrated plasma samples from 50 healthy donors.
The major plasma inhibitor of Kallikrein is C1-Inhibitor, although ATIII, α_2-Macroglobulin and Protein C Inhibitor (PCI-1) contribute.

HAZARD WARNING
All materials of human origin were tested and found negative for the presence of HBsAg, anti-HB core, HCV antibodies and anti-HIV antibody. However, as with all preparations of human origin, these products cannot be assumed to be free from infectious agents and suitable precautions should be taken in their use and disposal.

NOTE
The recommended incubation times and standard or test sample dilutions may vary between different batches of this kit owing to differences in the specific activity of some batches of reagents.

REFERENCES
1. Gallimore MJ. Chromogenic peptide substrate assays for determining components of the plasma kallikrein system. Scand J Clin Lab Invest 1985; 45: 127-132.
2. van der Graff F, Koedman JA, Bouma BN. Inactivation of kallikrein in human plasma. J Clin Invest 1983; 71: 149-158.
3. Lahiri B, Bagdasarian A, Mitchell B, et al. Antithrombin-heparin cofactor: an inhibitor of human plasma kallikrein. Arch Biochem Biophys 1976; 175: 737-744.
4. Harpel PC. Human plasma alpha 2-macroglobulin. An inhibitor of plasma kallikrein. J Exp Med 1970; 132: 329-352.
5. Meijers JCM, Kanters DHA, Vlooswijk RAA, van Erp HE, Hessing M, Bouma BN. Inactivation of human plasma kallikrein and factor XIa by protein C inhibitor. Biochemistry1988; 27: 4231-4237.
6. Gallimore MJ, Amundsen E, Larsbraaten M, Lyngaas K, Faried E. Studies on plasma inhibitors of plasma kallikrein using chromogenic peptide substrate assays. Thromb Res 1979; 16: 695-703.

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