CoaChrom® PKK

Chromogenic Assay for Prekallikrein

REF. COA0070

For Research Use Only
Not For Use in Diagnostic Procedures
For *in vitro* Use Only

This kit is designed for the measurement of plasma prekallikrein (PKK) in human plasma. Prekallikrein activator converts prekallikrein to plasma kallikrein, which is able to cleave a specific tri-peptide chromogenic substrate and liberate p-nitroaniline (pNA), which can be measured photometrically. The pNA concentration is directly proportional to the plasma kallikrein concentration.

REAGENTS

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

1. Chromogenic Kallikrein Substrate, 10 mL 1 vial

10 µmol/vial chromogenic substrate. Reconstitute with 5 mL distilled water, transfer to a suitable plastic bottle and dilute with a further 5 mL sterile distilled water. Stable for at least 6 months at 2-8°C if kept free from contamination.

2. Prekallikrein Activator, 5 mL 2 vials

Reconstitute with 5 mL distilled water. Stable for 8 hours at 2-8°C or 6 months if stored in aliquots at -20°C.

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, pH 8.0. Store at 2-8°C. Diluted buffer should be used within 24 hours.

4. Standard Plasma, 1 mL 1 vial

Add 1.0 mL distilled water, leave for 5 minutes at room temperature and then mix gently until completely dissolved. Stable for 8 hours at 2-8°C.

Reagents required, but not provided:

20% acetic acid or 2% citric acid. Plastic tubes for dilutions.

BLOOD COLLECTION AND PLASMA PREPARATION

Blood (9 mL) is mixed with 0.106 M Tri-sodium citrate (1 mL) and centrifuged at 2000g 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

The standard plasma is diluted with diluted buffer as follows:

Standard %	Plasma (µL)	Buffer (µL)
150	75	2500
100	50	2500
From the 100% standard prepare:		
75	600	200
50	400	400
25	200	600
0	Use buffer alone	

Dilute 50 µL of test plasma with 2500 µL diluted buffer.

ASSAY METHOD

Have the substrate and the activator at 37°C. Into siliconized semi-micro cuvettes, siliconized glass or plastic tubes pipette:

Plasma dilution or buffer	200 µL

Incubate at 37°C for 2 minutes, add:

Prekallikrein Activator	200 µL

Incubate at 37°C for 5 minutes, add:

Chromogenic Kallikrein Substrate	200 µL

Mix and record the change in optical density for a total of 5 minutes starting at 1 minute trough to 6 minutes at 405nm (kinetic method), or incubate for exactly 15 minutes at 37°C, add:

Acetic acid or Citric acid	200 µL

Mix and read optical density at 405 nm (endpoint method)

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.

Assay Blanks

Blanks should be prepared if the test plasmas have high bilirubin levels, are lipaemic or have visible haemolysis. For the blanks take 200 µL of diluted plasma, add 400 µL buffer and 200 µL acetic or citric acid (for the microtitre method, reduce these volumes by a factor of four).

CALCULATION

Subtract the blank values from the test values. Plot the results for A_{405} against percentage prekallikrein for the standard plasma dilutions and the values for the test plasma from the standard curve. The values can be expressed either as a percentage or in units per mL (U/mL) by applying the formula:

$$\text{PKK (U/mL)} = \frac{\text{\% Activity x Potency of Standard}^{*}}{100}$$

* value printed on the standard plasma vial label

PERFORMANCE CHARACTERISTICS

The standard curve is linear up to 150% (1.5 U/mL). The intra-assay coefficient of variation is 4% at 1.00 U/mL. The detection limit is 0.05 U/mL

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 standard and 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. Binnema DJ, Dooijewaard G, van Lersel JJL, Turion PNC, Kluft C. Thromb Haem 1990; 64: 390-397.
2. Adam A, Albert A, Boulanger J, Genot D, Demoulin A, Damas J. Clin Chem 1985; 31: 1533-1536.

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