CoaChrom® KKA

Chromogenic assay for Kallikrein-Like Activity
Art.No. COA0087

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

This kit is designed for the determination of kallikrein-like activity in human plasma1,2. This activity is predominantly due to kallikrein bound to alpha-2-macroglobulin. Plasma is diluted with buffer and kallikrein-like activity is measured using a chromogenic peptide substrate for plasma kallikrein. Cleavage of the substrate liberates p-nitroaniline (pNA), which can be measured photometrically. Kallikrein-like activity can be calculated from the amount of pNA released.

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

1. Chromogenic Kallikrein Substrate, 10 mL 1 vial
10 µmol/vial of MBz-Pro-Phe-Arg-pNA. reconstitute with 5 mL sterile distilled water, transfer to a suitable plastic vial 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. High Activity Standard, 0.5 mL 1 vial
Reconstitute with 0.5 mL distilled water, leave for 5 minutes at room temperature and then mix gently until completely dissolved. Stable at room temperature for 4 hours, do not refrigerate.

3. Low Activity Standard, 1.0 mL 1 vial
Reconstitute with 1.0 mL distilled water, leave for 5 minutes at room temperature and then mix gently until completely dissolved. Stable at room temperature for 4 hours, do not refrigerate.

4. Buffer Concentrate, 10 mL 2 vials
Dilute the buffer concentrate 1+9 with distilled water. This gives an assay buffer of 0.05M Tris-HCl, 0.1M NaCl, pH 8.0. Store at 2-8°C. Diluted buffer should be used within 24 hours.

Reagents and materials required, but not provided
20% acetic acid or 2% citric acid; 10 mL plastic tube for substrate dilution.

BLOOD COLLECTION AND PLASMA PREPARATION
Blood (9 mL) is mixed with 0.106M 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.

Note: in plasma samples containing heparin, the heparin must be neutralised with protamine sulphate or protamine chloride before freezing (1 mg protamine neutralises approximately 100 IU heparin).

STANDARD AND TEST DILUTIONS
Dilute 100 µL of High Activity Standard Plasma, Low Activity Standard Plasma and test plasmas with 1000 µL diluted buffer in plastic tubes at room temperature.

ASSAY METHOD
Have the Substrate at 37°C. Into plastic tubes pipette:

Sample or Standard dilution	400 µL

Mix and incubate at 37°C for 3 minutes, add:

Chromogenic Substrate	200 µL

Mix and record the change in optical density per minute at 405 nm (rate assay), or incubate for exactly 20 minutes at 37°C, add:

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

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

Microtitre method
Follow the manual method above but pipette the following volumes into the wells of a microtitre plate: using 100 µL sample dilution and 50 µL Chromogenic Substrate and Acetic acid or Citric acid. Care must be taken to ensure adequate mixing after each reagent addition.

CALCULATION
With the end point assay, if the test plasmas have high Bilirubin levels, are lipaemic or have visible

haemolysis, blanks must be performed. For blanks add the reagents without incubation in the reverse order and substitute buffer for the substrate. The A_{405} values for the blanks are subtracted from the test values.

Multiply the optical density values by 164; this gives kallikrein-like activities in U/L.
For microplate assays, use a multiplication factor of 282 to obtain U/L.

INTERPRETATION
The Low Activity Standard Plasma gives an activity similar to a plasma where low kallikrein-like activities are present. The High Activity Standard Plasma gives high kallikrein-like activity. Plasma samples from normal subjects should give a value close to that for the Low Activity Standard Plasma. Plasma samples from patients in whom plasma kallikrein has been activated will give values higher than the value for the Low Activity Standard Plasma.

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 the reagents.

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