CoaChrom® HMW-Kininogen

Chromogenic Assay for High Molecular Weight Kininogen

Art.No. COA0141

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

This kit is designed for the measurement of High Molecular Weight Kininogen (HMW-Kininogen) in plasma. Plasma is diluted in buffer and mixed with HMW-Kininogen deficient plasma. Activator reagent is added and converts Prekallikrein to Kallikrein, which in turn activates Factor XII. Calcium chloride, a synthetic peptide Thrombin inhibitor, and a chromogenic substrate for Factor Xa are added. The activated Factor XII causes sequential activation of Factor XI, Factor IX, and Factor X. Factor Xa is able to cleave the specific chromogenic substrate and liberate p-nitroaniline (pNA), which can be measured photometrically. The pNA concentration is directly proportional to the plasma HMW-Kininogen concentration.

REAGENTS

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

1. Chromogenic Factor Xa Substrate, 2.5 mL 1 vial
CH_3SO_2-D-But-Gly-Arg-pNA and Thrombin inhibitor. Reconstitute with 2.5 mL distilled water. Stable for 8 hours at 2-8°C or 6 months at -20°C. Mix well after thawing.

2. Calcium Chloride, 2.5 mL 1 vial
0.05 mol/L. Stable for 1 week at 2-8°C.

3. HMW-Kininogen Activator, 2.5 mL 1 vial
A soluble activator of plasma Prekallikrein. Reconstitute with 2.5 mL distilled water, leave for 10 minutes at room temperature. Mix well before use. Stable for 8 hours at 2-8°C. DO NOT FREEZE.

4. HMW-Kininogen Deficient Plasma, 2.5 mL 1 vial
Add 2.5 mL distilled water, leave for 10 minutes at room temperature and then mix gently until completely dissolved. Stable for 8 hours at 2-8°C or 3 months at -20°C. If frozen, thaw at 37°C and mix before use.

5. Standard Plasma, 1.0 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.

6. 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, 0.15M NaCl, pH 8.0. Store at 2-8°C. Diluted buffer should be used within 24 hours.

Reagents required, but not provided: 20% acetic acid or 2% citric acid.

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.

PREPARATION OF THE STANDARD CURVE

The standard plasma is diluted with assay buffer as follows:

Standard Plasma (µL)	Buffer (µL)	HMW-K Act. (%)
12.5	1988	125
10.0	1990	100

Dilute 100% Standard as follows to give:

100% Std. (µL)	Buffer (µL)	HMW-K Act. (%)
75	25	75
50	50	50
25	75	25
Use assay buffer alone		0

Dilute 12.5 µL of each test plasma (sample) with 1987 µL assay buffer.

MICROTITRE ASSAY METHOD

Mix equal volumes of the chromogenic substrate and calcium chloride (e.g. 1 mL + 1 mL). Warm and maintain the mixture at 37°C.

Into the wells of a microtitre plate, pipette:

Diluted plasma or assay buffer (blank)	25 µL
HMW-Kininogen Deficient Plasma	25 µL

Incubate at 37°C for 2 minutes, add:

HMW-Kininogen Activator	25 µL

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

Substrate/Calcium Chloride Mixture (37°C)	50 µL

Mix and incubate for exactly 30 minutes at 37°C, add:

Acetic acid (20%) or citric acid (2%)	50 µL

Mix and read optical density at 405nm.

CALCULATION

Prepare blanks exactly as the test or standard samples, substituting 25 µL assay buffer for the HMW-Kininogen Activator. Assay as for test samples. Subtract the A_{405} values for the blanks from the A_{405} values for the test samples and plot the corrected absorbance values for the standards against log HMW-Kininogen activity (%). Read the values for the test plasmas from the curve.

Samples with HMW-Kininogen levels above 125% must be re-assayed after diluting 1:1 with assay buffer and multiplying the HMW-Kininogen value by factor 2.

The values can be expressed either as a percentage or in units per mL (U/ml) by applying the formula:

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

* value printed on the standard plasma vial label.

PERFORMANCE CHARACTERISTICS

The standard curve is linear up to 125%. The intra-assay coefficient of variation is 5% at 1.00 U/mL (100%).

INTERPRETATION

Normal Range 0.81 - 1.29 U/ml (81-129%)

The assay may be affected by heparin, which will potentiate inhibition. If samples containing heparin must be assayed, the heparin should first be removed or neutralised.

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.

If absorbance will be measured using a cuvette spectrophotometer, increase the reagent volumes proportionally to give a final volume suitable for the instrument and cuvettes used.

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