CoaChrom® Factor XIIa

Chromogenic Assay for Factor XIIa-like Activity

REF. COA0088

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

This kit is designed for the measurement of Factor XIIa-like activity in human plasma. FXIIa-like activity is predominantly the activity of αFXII bound to α-2-Macroglobulin. Plasma is diluted with buffer and FXIIa-like activity is measured using a chromogenic substrate. Cleavage of the substrate liberates para-nitroaniline (pNA), which can be measured photometrically. FXIIa-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 Factor XIIa Substrate, 10 mL 1 vial
10 µmol 2AcOH.H-D-CHT-Gly-Arg-pNA. Reconstitute with 10 mL distilled water. Stable for at least 6 months at 2-8°C if kept free from contamination.

2. Kallikrein Inhibitor, 10 mL 1 vial
Soybean Trypsin Inhibitor and buffer salts. Reconstitute with 10 mL distilled water. Stable for 8 hours at 2-8°C or 6 months at -20°C.
Before use dilute 1 mL with 49 mL assay buffer.

3. 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, pH 7.9. Store at 2-8°C. Diluted buffer should be used within 24 hours.

4. Standard Plasma "High", 0.5 mL 1 vial
Add 0.5 mL distilled water, leave for 5 minutes at room temperature and then mix gently until completely dissolved. Stable for 4 hours at 18-25°C, do not refrigerate.

5. Standard Plasma "Low", 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 4 hours at 18-25°C, do not refrigerate.

Reagents required, but not provided

20 % acetic acid or 2 % citric acid.

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.

STANDARD AND TEST DILUTIONS

Dilute 100 µL of high activity standard plasma (4), low activity standard plasma (5) and test plasmas with 1000 µL assay buffer containing kallikrein inhibitor (2), in plastic tubes at room temperature.

ASSAY METHOD

Have the substrate (1) at 37°C and keep the plasma dilutions at room temperature. Into siliconised semi-micro cuvettes, siliconised glass or plastic tubes pipette:

Plasma dilution	400 µL
Mix and incubate at 37°C for 3 minutes, add:	
Chromogenic Substrate (1)	200 µL
Mix and incubate at 37°C for 30 minutes, add:	
Acetic acid (20%) or citric acid (2%)	200 µL
Mix immediately.	

Prepare plasma blanks by adding the reagents in reverse order without incubation, substituting buffer for substrate. Read the absorbance of the test samples and blanks in a spectrophotometer at 405 nm. Subtract the absorbance values for the blanks from the test values.

Microplate method

Follow the manual method above but pipette the following volumes into the wells of a microtitre plate: using 100 µL plasma dilution, 50 µL substrate and 50 µL acetic acid or citric acid. Care must be taken to ensure adequate mixing after each reagent addition.

CALCULATION

Multiply the optical density values by **147**, this gives FXIIa-like activities in **U/L**. For microplate assays, use a multiplication factor of **253** to obtain **U/L**.

INTERPRETATION

The low activity standard plasma gives an activity

similar to a plasma where low FXIIa-like activities are present. The high activity standard plasma gives high FXIIa-like activity. Plasma samples from normals should lie near to the value for the low activity standard. Plasma samples from patients in whom FXII has been activated will give values higher than the value for the low activity standard.

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. Kluft C et al. Semin Thr.Hemost 1987; 13: 50-68
2. Mannhalter C et al. Fibrinolysis 1987; 1: 259-263
3. Laemmle B et al. Thr Haemost 1991; 65: 117-121

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