CoaChrom® α-FXIIa Inhibitor

Chromogenic assay for α-Factor XIIa Inhibitors

Art.No. COA0045

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

This kit is designed for the determination of α-Factor XIIa (α-FXIIa) Inhibitors in human plasma. Purified α-FXIIa is added to diluted plasma and a proportion of the enzyme complexes to its plasma inhibitors. The residual α-FXIIa activity is then measured using a chromogenic peptide substrate. The concentration of pNA cleaved from the substrate is measured photometrically and is inversely proportional to the concentration of α-FXIIa inhibitors.

REAGENTS

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

1. Chromogenic Factor XIIa Substrate, 10 mL 1 vial
10 µmol of H-D-CHT-Gly-Arg-pNA plus mannitol. Reconstitute with 10 mL sterile distilled water. Stable for at least 6 months at 2-8°C if kept free from contamination.

2. α-Factor XIIa, 10 mL 1 vial
Reconstitute with 10 mL distilled water. Stable for 4 hours at 2-8°C or 6 months at -80°C if snap frozen immediately after reconstitution, thaw for 5 minutes at 37°C. A small loss of activity may occur on freezing and thawing.

3. Kallikrein Inhibitor, 10 mL 1 vial
Soybean Trypsin Inhibitor and buffer salts. Reconstitute with 10 mL distilled water. Stable for 24 hours at 2-8°C or 6 months at -20°C.
3a. Before use dilute 1 mL with 49 mL assay buffer. Dilution stable for 8 hours at 2-8°C.

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, pH 7.8. Store at 2-8°C. Diluted buffer should be used within 24 hours.

5. 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 and materials 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.

STANDARD AND SAMPLE DILUTIONS

Dilute the Standard Plasma in Kallikrein Inhibitor working solution (KKI WS, 3a) as follows:

Standard (%)	*Plasma (µL)*	*KKI WS (3a) (µL)*
150	12.50	1487.50
100	12.50	1987.50
From the 100% Standard prepare:		
75	300	100
50	200	200
25	100	300
0	Use KKI working solution (3a) alone	

Dilute 12.50 µL of each sample plasma with 1987.50 µL KKI working solution (3a).

ASSAY METHOD

Have the Substrate (1) at 37°C. Into siliconised semi-micro cuvettes, siliconised glass or plastic tubes pipette:

Plasma dilutions or KKI working solution (3a) 200 µL

Mix and incubate at 37°C for 2 minutes, add:

α-Factor XIIa (2) 200 µL

Mix and incubate at 37°C for 15 minutes, add:

Chromogenic Factor XIIa Substrate (1) 200 µL

Mix and record the change in optical density per minute at 405 nm (kinetic method), or incubate for exactly 30 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 50 µL

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

With the end point assay, if the test plasmas have high Bilirubin levels, are lipaemic or have visible haemolysis, blanks must be performed. For the blanks add the reagents in the reverse order and substitute buffer for the FXIIa substrate and for α-FXIIa. The A_{405} values for the blanks are subtracted from the test values before reading the α-FXIIa-Inhibitor values from the standard curve.

Plot the results as A_{405} against percentage α-FXIIa-Inhibitor for the standard plasma dilutions and read the values for the test plasmas from the curve. The values can be expressed either as a percentage or in units per ml (U/ml) by applying the formula:

α-FXIIa-Inhibitor (U/mL) =

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

* value printed on the standard plasma vial label.

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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