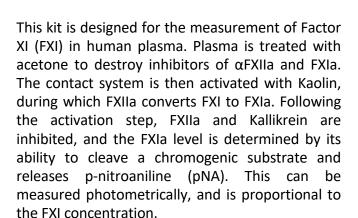
CoaChrom® Factor XI

Chromogenic Assay for Factor XI

Art.No. COA0090

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



REAGENTS

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

1. Chromogenic Factor XIa Substrate, 10 mL 1 vial 10 μmol/vial 2AcOH.H-D-Lys(Cbo)-Pro-Arg-pNA. Reconstitute with 10 mL sterile distilled water. Stable for at least 6 months at 2-8°C if kept free from contamination. It may also be stored in aliquots at below -20°C.

2. Kaolin, 10 mL 1 vial

Suspend in 10 mL assay buffer, shake well before use. Stable for 6 months at 2-8°C.

3. Buffer Concentrate, 10 mL 2 vials Dilute the buffer concentrate 1+9 with distilled water. This gives an assay buffer of 0.02M Tris-HCl, 0.15M NaCl, 0.001M Na₂EDTA, pH 7.4. Store at 2-8°C. Diluted buffer ("assay buffer") should be used within 24 hours.

4. Kallikrein Inhibitor, 10 mL 1 vial Recunstitute with 10 mL distilled water and dilute 1 mL with 99 mL assay buffer. Stable for 8 hours at 2-8°C or 6 months at -20°C.

5. Corn Trypsin Inhibitor, 10 mL 1 vial Reconstitute with 10 mL distilled water. Stable for 8 hours at 2-8°C or 6 months at -20°C.



6. Standard Plasma, 1 mL

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, Acetone.

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.

ACETONE TREATMENT OF PLASMA

600 μL Standard plasma (or 300 μL test plasma) and 200 µL acetone (100 µL acetone for test plasmas) are pipetted into siliconised glass test tubes (80x10mm), or plastic tubes, mixed well and left for 15 minutes at 2-8°C, then kept on ice until assayed.

PREPARATION OF THE STANDARD CURVE

The acetone treated standard plasma is diluted with buffer as follows:

Standard %	Plasma	Buffer
150	150 μL	850 μL
100	200 μL	1800 μL
From the 100% Standard prepare:		
75	600 μL	200 μL
50	400 μL	400 μL
25	200 μL	600 μL
0	Use buffer alone	

Dilute 100 µL of each acetone treated test plasma with 900 µL assay buffer.

ASSAY METHOD

Have the Substrate at 37 °C, shake the Kaolin well

before use. Into siliconised semi-micro cuvettes, siliconised glass or plastic tubes pipette:

Plasma dilution or buffer 200 μL

Kallikrein Inhibitor 200 μL

Mix and incubate at 37 $^{\circ}$ C for 2 minutes, add:

Kaolin 200 μL

Mix well, incubate for 60 minutes at 37 $^{\circ}$ C, add:

Corn Trypsin Inhibitor 200 μL

Mix well, incubate for 10 minutes at 37 $^{\circ}$ C, add:

FXIa Chromogenic Substrate 200 μL

Mix and record the change in optical density per minute at 405nm (rate assay), or incubate for exactly 60 minutes at 37 $^{\circ}$ C, add:

Acetic acid or Citric acid 200 µL

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

MICROTITRE METHOD

Follow the manual method above, but pipette $30~\mu L$ volumes 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 200 μL of buffer instead of FXIa Substrate. The A_{405} values for the blanks are subtracted from the test values before reading the Factor XI values from the standard curve.

Plot the results as A_{405} against percentage FXI for the standard plasma dilutions and read 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:

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

PERFORMANCE CHARACTERISTICS

The standard curve should be linear up to 150%. Intra-assay CV: <5% at 1 U/mL. Detection limit: 5%.

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.



CoaChrom Diagnostica GmbH

Hauptstrasse 5, 2344 Maria Enzersdorf, Austria info@coachrom.com www.coachrom.com T: +43-1-236 222 1 F: +43-1-236 222 111 Toll free from Germany:

T: 0800 - 246 633 0 F: 0800 - 246 633 3

^{*} value printed on the standard plasma vial label