CoaChrom® Factor XIa Inhibitors

Chromogenic Assay for Inhibitors of Factor XIa

ART.NO. COA0091

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

This kit is designed for the determination of inhibitors of Factor XIa (FXIa-I) in human plasma. Plasma is diluted in buffer containing an inactivator of α_2-macroglobulin. Excess FXIa is added and during an incubation period it complexes with plasma inhibitors. The remaining free FXIa is measured by its ability to cleave a chromogenic peptide substrate and liberate p-nitroaniline (pNA). This can be measured photometrically, and the absorbance is inversely proportional to the plasma inhibition of FXIa.

REAGENTS

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

1. Chromogenic FXIa Substrate, 10 mL 1 vial
10 µmoL/vial 2AcOH.H-D-Lys(Cbo)-Pro-Arg-pNA.
Reconstitute with 10 mL distilled water. Stable for at least 6 months at 2-8°C if kept free from contamination.

2. Human FXIa, 10 mL 1 vial
Reconstitute with 10 mL distilled water.
Stable for 6 hours at 2-8°C or 6 months at ≤ -70°C if it is snap frozen immediately after reconstitution, although a small loss of activity may be found on freezing and thawing.

3. Human Albumin, 10 mL 1 vial
Reconstitute with 10 mL distilled water. Stable for 8 hours at 2-8°C or 6 months at -20°C.

4. Buffer Concentrate, 10 mL 2 vials
Dilute 1 volume of buffer concentrate with 8.5 volumes of distilled water and add 0.5 volumes human albumin (Reagent 3), e.g. 10 mL buffer concentrate, 85 mL water and 5 mL human albumin. This gives an assay buffer of 0.05 M Tris-HCl, 0.4 M NaCl, 0.5 % albumin, pH 8.0, containing an inhibitor of α_2-macroglobulin. 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 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 PLASMA DILUTIONS

A) Dilute Standard Plasma (Reagent 5) with diluted buffer as follows:

Standard %	*Plasma*	*Buffer*
150	150 µL	850 µL
100	100 µL	900 µL
From the 100 % Standard prepare:		
75	300 µL	100 µL
50	200 µL	200 µL
25	100 µL	300 µL
0	Use buffer alone	

B) Dilute 100 µL of each test plasma with 900 µL buffer.

ASSAY METHOD

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

Plasma dilution or buffer 200 µL

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

Human FXIa (Reagent 2) 200 µL

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

Chromogenic FXIa Substrate (Reagent 1) 200 µL

Mix and record the optical density change for a total of 5 minutes starting at 1 minute through to 6 minutes at 405 nm (rate assay), or incubate for exactly 8 minutes at 37°C, add:

Acetic acid or Citric acid 200 µL

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

Microtitre method
Follow the manual method above, but pipette 50 µL 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 take 200 µL of diluted plasma, add 400 µL of buffer and 200 µL acetic acid or citric acid. The A_{405} values for the blanks are subtracted from the test values before reading the FXIa-I values from the standard curve.
Plot the results as A_{405} against percentage FXIa-I 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:

FXIa Inhibitors (U/mL) =

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

*according to the value printed on the standard vial label

PERFORMANCE CHARACTERISTICS
The standard curve is linear up to 150 %. Intra-assay CV: <5 % at 1U/ml. Detection limit: 5 %.

INTERPRETATION
A 2 standard deviation range of 0.70 - 1.50 U/mL (70-150 %) was obtained using citrated plasma samples from 50 healthy normal subjects.
The major plasma inhibitors of FXIa are C1-esterase inhibitor, α1-antitrypsin, α2-antiplasmin, ATIII and protein C inhibitor (PCI-1).

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.

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