

CoaChrom[®] Factor X

Chromogenic Assay for Factor X
Art.No. 400002



For in vitro research use only

This kit is designed for the measurement of Factor X (FX) in human plasma. Factor X is converted to FXa by an enzyme from the venom of Russell's viper (RVV). The active protease, FXa, cleaves a chromogenic substrate and liberates p-nitroaniline (pNA), which can be measured photometrically. The influence of any trace amounts of thrombin that may be generated are blocked by a synthetic thrombin inhibitor. The amount of pNA liberated is directly proportional to the FX concentration.

REAGENTS

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

1. Chromogenic Factor Xa Substrate, 10 mL 1 vial
10 µmol H-D-CHT-Gly-Arg-pNA chromogenic Factor Xa substrate, thrombin inhibitor and mannitol. Reconstitute with 10 mL aqua dest. Stable for at least 6 months at 2-8°C.

2. Russell's Viper Venom (RVV), 5 mL 1 vial
0.05 mg RVV with buffer salts and stabilizers. Reconstitute with 5.0 mL aqua dest. Stable for 8 hours at 2-8°C or 6 months at -20°C.

3. Buffer Concentrate, 10 mL 2 vials
Dilute the buffer concentrate 1+9 with aqua dest. This gives an assay buffer of 0.05M Tris-HCl, 0.23M NaCl, pH 8.3. Diluted assay buffer should be used within 24 hours.

4. Calcium Chloride, 5 mL 1 vial
0.05M, ready to use. Once opened stable for 6 months at 2-8°C.

5. Standard Plasma, 1 mL 1 vial
Add 1.0 mL aqua dest, leave for 5 minutes at room temperature and then mix gently until dissolved. The labelled potency is assigned against the 3rd International Standard for Blood Coagulation Factors II and X 98/590. Stable for 8 hours at 2-8°C or 6 months at -20°C.

Reagents required, but not provided

20% acetic acid or 2% citric acid.

Preparation of RVV/CaCl₂ mixture

Mix equal volumes of RVV (Reagent 2) and Calcium Chloride (Reagent 4).

BLOOD COLLECTION AND PLASMA PREPARATION

Blood (9 parts) is mixed with 0.106 M Tri-sodium citrate (1 part) and centrifuged at 2000 g 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 (Reagent 5) is diluted with assay buffer as follows:

Standard (%)	Plasma (µL)	Buffer (µL)
150	75	1925
100	50	1950

From the 100% Standard, prepare:

75	600	200
50	400	400
25	200	600
0	Use assay buffer alone	

SAMPLE PRE-DILUTION

Dilute 25 µL of each test plasma with 975 µL of assay buffer.

ASSAY PROTOCOL

Have the substrate at 37°C and RVV/CaCl₂ at ambient temperature. Into siliconised semi-micro cuvettes, siliconised glass or plastic tubes pipette:

Plasma dilution or buffer 400 µL

Incubate at 37°C for 2 minutes, add:

RVV/CaCl₂ 200 µL

Incubate at 37°C for exactly 5 minutes, add:

Chromogenic Substrate 200 µL

Mix and record the change in optical density per minute at 405 nm (rate assay), or incubate for exactly 10 minutes at 37 °C, add:

Acetic acid or citric acid 200 µL

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

Microplate Method

Follow the manual method above, but pipette 100 µL of each plasma dilution and 50 µL of each reagent into the wells of a polystyrene microplate. 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, substitute 200 µL assay buffer for the Factor X substrate and 200 µL assay buffer for RVV/CaCl₂ (for the microplate method, reduce these volumes by a factor of four). The A₄₀₅ values for the blanks are subtracted from the test values before reading the Factor X values from the standard curve.

Plot the results as A₄₀₅ against percentage factor X 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 (IU/mL) by applying the formula:

$$\text{FX (IU/mL)} = \frac{\% \text{ Activity} \times \text{Potency of Standard}}{100}$$

PERFORMANCE CHARACTERISTICS

The standard curve is linear up to 1.5 IU/mL (150%). The detection limit is 0.01 IU/mL (1%). Intra-assay CV <3% at 1 IU/mL and <2.7% at 0.33 IU/mL.

INTERPRETATION

Normal Range 0.50 - 1.50 IU/mL.

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 incubation times and standard or test sample dilutions may vary between different batches of this kit owing to differences in the specific activity of some batches of reagents.

REFERENCES

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