CoaChrom® Factor XII

Chromogenic assay for Factor XII

Art.No. COA0068

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

This kit is designed for the measurement of Factor XII (FXII, Hageman factor) in plasma. Factor XII is converted to FXIIa with an activator. The active protease FXIIa cleaves a chromogenic substrate and releases p-nitroaniline (pNA), which can be measured photometrically.

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. Reconstitute with 5 mL sterile distilled water, transfer to a suitable plastic vial and dilute with a further 5 mL sterile distilled water. Stable for at least 6 months at 2-8°C if kept free from contamination.

2. Factor XII Activator, 5 mL 1 vial
A mixture of a soluble surface activator and a plasma fraction containing Prekallikrein, HMW-Kininogen and stabiliser. Reconstitute with 5.0 mL distilled water. Stable for 8 hours at 2-8°C or 6 months at -20°C.

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; acetone; 10 mL plastic tube for substrate dilution.

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, or plastic tubes, mixed well and left for 15 minutes at 2-8°C, then kept on ice until assayed.

STANDARD AND TEST DILUTIONS

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

Standard (%)	*Plasma (µL)*	*Buffer (µL)*
150	75	325
100	50	350
75	37.5	363
50	25	375
25	12.5	388
0	Use buffer alone	

<u>Dilute 50 µL of each acetone treated sample plasma with 350 µL assay buffer.</u>

ASSAY METHOD

Have the Substrate and the diluted Kallikrein Inhibitor (3a) at 37°C. Into siliconised semi-micro cuvettes, siliconised glass or plastic tubes pipette:

Plasma dilution or buffer	100 µL
Factor XII Activator	100 µL
Mix and incubate at 37°C for 10 minutes, add:	
Kallikrein Inhibitor	300 µL
Incubate for exactly 1 minute, add:	
Chromogenic Substrate	200 µL

Mix and record the change in optical density per

minute at 405 nm (rate assay) for a total of 5 minutes starting at 1 minute through 6 minutes, 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 the following volumes into the wells of a microtitre plate: using 30 µL plasma dilution, 30 µL FXII Activator, 90 µL Kallikrein Inhibitor (3a), 60 µL Chromogenic Substrate and 60 µL Acetic acid or Citric acid. 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 FXII substrate and for FXII Activator. The A_{405} values for the blanks are subtracted from the test values before reading the FXII values from the standard curve.

Plot the results as A_{405} against percentage FXII 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:

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

* value printed on the standard plasma vial label.

PERFORMANCE CHARACTERISTICS

The standard curve is linear up to 150%. Intra-assay CV: 5.5% at 100% (1.00 U/mL). Detection limit: 5%.

INTERPRETATION

Normal Range 0.50 - 1.45 U/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 standard and test sample dilutions may vary between different batches of this kit, owing to differences in the specific activity of the reagents.

REFERENCES

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