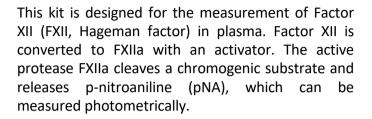
CoaChrom® Factor XII

Chromogenic assay for Factor XII

Art.No. COA0068

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



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

Dilute 50 µL of each acetone treated sample plasma with 350 μL assay buffer.

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 սե

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 μ L plasma dilution, 30 μ L FXII Activator, 90 μ L Kallikrein Inhibitor (3a), 60 μ L Chromogenic Substrate and 60 μ L 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 A₄₀₅ 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:

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

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

- 1. Walshe K et al. Thromb Res 1987; 47: 365-371.
- 2. Velasco F et al. Thr Haem 1986; 55: 357-360.
- 3. Martinez-Brotons F et al. Th H 1987; 58: 709-713.
- 4. Mason JN & Colman RW. Thromb Diath Haem 1971; 26: 325.
- 5. Kluft C et al. Semin Thr Haem 1987; 13: 50-68.
- 6. Goodnough LT et al. Medicine 1983; 62: 248-255.
- 7. Mannhalter C et al. Fibrinolysis 1987; 1: 259-263.
- 8. Lammle B et al. Thr Haem 1991; 65: 117-121.
- 9. Gorden EM et al. J Lab.Cl.M. 1980; 96: 781-790.
- 10. Jespersen J, Kluft C. Thr Haem 1985; 54:454-459.



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