CHROMOGENIC SUBSTRATE ASSAY FOR FACTOR XII

This kit is designed for the measurement of factor XII (FXII. Hageman factor) in human plasma. Factor XII is converted to FXIIa with an activator preparation, and the active protease FXIIa cleaves a chromogenic substrate and liberates p-nitroaniline (pNA), which can be measured photometrically[1,2].

REAGENTS

The reagents should be stored at 4°C until reconstituted.

1. *Factor XII Substrate*
10µmol/vial 2AcOH.H-D-CHT-Gly-Arg-pNA, plus mannitol. Dissolve in 10ml sterile distilled water. Stable for at least 6 months at 4°C if kept free from contamination. It may also be stored in aliquots at below -20°C.

2. *Factor XII Activator*
Dissolve in 5ml distilled water. Stable for 8 hours at 4°C and 6 months at -20°C.

3. *Buffer Concentrate*
Dilute the vial contents (10ml) with 90ml distilled water. This gives a buffer of 0.05M Tris-HCl, pH 7.9. Store at 4°C.

4. *Kallikrein Inhibitor*
Dissolve in 10ml distilled water and dilute 1ml with 49ml assay buffer. Stable for 8 hours at 4°C and 6 months at -20°C.

5. *Standard Plasma*
Add 1.0ml distilled water, leave for 5 minutes at room temperature and then mix gently until completely dissolved. Stable for 8 hours at 4°C.

Reagents required, but not provided: 50% acetic acid; acetone.

BLOOD COLLECTION AND PREPARATION OF PLASMA

Blood (9ml) is mixed with 0.106M Tri-sodium citrate (1ml) 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

600µl Standard plasma (or 300µl test plasma) and 200µl acetone (100µl acetone for test plasmas) are pipetted into siliconised glass test tubes (80x10mm), or polypropylene tubes, mixed well and left for 15 minutes at 4°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 (µl)	Buffer (µl)
150	75	325
100	50	350
75	37.5	363
50	25	375
25	12.5	388
0	Use buffer alone	

Dilute 50µl of each acetone treated test plasma with 350µl buffer.

ASSAY METHOD

Have the substrate and buffer containing kallikrein inhibitor at 37°C. Into siliconised semi-micro cuvettes, siliconised glass or plastic tubes pipette:

Plasma dilution or buffer	100µl
Add Factor XII Activator	100µl

Mix and incubate at 37°C for 10 minutes, add:

Kallikrein Inhibitor	300µl

Incubate for exactly 1 minute, add:

Factor XII Substrate	200µl

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

Acetic acid (50%)	200µl

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

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 200µl buffer for the Factor XII substrate and 100µl buffer for Factor XII Activator. The A405 values for the blanks are subtracted from the test values before reading the Factor XII values from the standard curve.

Plot the results as A405 against percentage factor XII 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:

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

The potency of the standard plasma for FXII (lot UD-0137-0497) is 1.00 U/ml.

PERFORMANCE CHARACTERISTICS

The standard curve should be linear up to 150%.
Intra assay cv = 5.5% at 1U/ml.

INTERPRETATION

Normal Range 0.70 - 1.45 U/ml

Factor XII levels may be reduced in congenital deficiency as well as in multi-systems organ failure, sepsis, and DIC[4,5]. Factor XII is a component of the contact factor mediated intrinsic pathway of fibrinolysis[6] and deficiency may be associated with an increased risk of venous thromboembolism[7,8]. Factor XII levels may be increased in women taking combined oral contraceptives[9,10,11].

HAZARD WARNING

All materials of human origin were tested and found negative for the presence of HBsAG 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.

REFERENCES

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