CHROMOGENIC SUBSTRATE ASSAY FOR INHIBITORS OF FXIa

This kit is designed for the determination of inhibitors of factor XIa (FXIa) 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 4°C until reconstituted.

1. *Factor XIa Substrate*
10µmol/vial 2AcOH.H-D-Lys(Cbo)-Pro-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 -20°C.

2. *Human FXIa*
Reconstitute in 10ml sterile distilled water.
Stable for 6 hours at 4°C and 6 months at -20°C.

3. *Human Albumin*
Dissolve in 10ml distilled water. Stable for 8 hours at 4°C and 6 months at -20°C.

4. *Buffer Concentrate*
Dilute 10ml of buffer concentrate with 85ml sterile distilled water, and add 5ml Human Albumin. This gives a buffer of 0.05M Tris-HCl, 0.4M NaCl, 0.5% albumin, pH 8.0, containing an inhibitor of α_2-macroglobulin. store at 4°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.

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.

PREPARATION OF THE STANDARD CURVE

Dilute 100µl Standard Plasma with 900µl buffer and then further dilute as follows:

STANDARD %	PLASMA	BUFFER
150	25µl	1475µl
100	25µl	1975µl
From the 100% Standard prepare:		
75	300µl	100µl
50	200µl	200µl
25	100µl	300µl
0	Use buffer alone	

Dilute 100µl of each test plasma with 900µl buffer, then further dilute 25µl of this with 1975µl buffer.

ASSAY METHOD

Have the FXIa Substrate at 37°C.

Into siliconised semi-micro cuvettes, siliconised glass or plastic tubes pipette:

Plasma dilution or buffer	200µl

Incubate at 37°C for 2 minutes, add:

FXIa	200µl

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

FXIa Substrate	200µl

Mix and record the change in optical density per minute at 405nm (rate assay), or incubate for exactly 30 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 take 200µl volumes of diluted plasma, add 400µl buffer and 200µl acetic acid and mix. The A_{405} values for the blanks are subtracted from the test values before reading the FXIa inhibition values from the standard curve.

Plot the results as A_{405} against percentage FXIa inhibition for the standard plasma dilutions and read the values for the test plasmas from the standard curve. The values can be expressed either as a percentage or in units per ml (U/ml) by applying the formula:

$$\text{FXIa Inhibitors (U/ml)} = \frac{\%\ \text{Activity} \times \text{Potency of Standard}}{100}$$

The potency of the standard plasma for FXIa Inhibitors (lot 0137-0316) is 1.25 U/ml.

PERFORMANCE CHARACTERISTICS

The assay is linear up to 150%, with a sensitivity limit of 5%. The intra-assay coefficient of variation is 5% at 1.00 U/ml.

INTERPRETATION

Normal Range 0.70 - 1.50 U/ml (70-150%)

The major plasma inhibitor of FXIa is α_1-antitrypsin[1], although AT-III[2] and protein C inhibitor[3] (PCI-1) contribute.

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

1. Heck LW & Kaplan AP. Substrates of Hageman factor. I. Isolation and characterization of human factor XI (PTA) and inhibition of the activated enzyme by α-antitrypsin. J Exp Med 1974; 140: 1615.

2. Scott CF, Schapira M, James HL, Cohen AB, Colman RW. The inactivation of factor XIa by plasma protease inhibitors. J Clin Invest 1982; 69: 844.

3. Meijers JCM, Kanters DHA, Vlooswijk RAA, van Erp HE, Hessing M, Bouma BN. Inactivation of human plasma kallikrein and factor XIa by protein C inhibitor. Biochemistry1988; 27: 4231-4237.

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