CHROMOGENIC SUBSTRATE ASSAY FOR DETERMINATION OF αFACTOR XIIa INHIBITORS

This kit is designed for the determination of αFactor XIIa (αFXIIa) Inhibitors in human plasma. Purified αFXIIa is added to dilute plasma and a proportion of the enzyme complexes to its plasma inhibitors. The residual αFXIIa activity is then measured using a chromogenic peptide substrate. The concentration of pNA cleaved from the substrate is measured photometrically and is inversely proportional to the concentration of αFXIIa inhibitors.

REAGENTS

The kit contents should be stored at 4°C until reconstituted.

1. *αFactor XIIa*
Dissolve in 10ml sterile distilled water.
Store at 4°C for up to 6 hours or at -20°C.

2. *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 at -20°C.

3. *Kallikrein Inhibitor*
Dissolve the contents of 1 vial in 10ml distilled water. Store at 4°C until required; store excess at -20°C.

4. *Buffer Concentrate*
Dilute the vial contents (10ml) with 90ml distilled water. This gives a buffer of 0.05M Tris-HCl pH 7.9.
Buffer Plus Kallikrein Inhibitor: Mix 1ml Kallikrein Inhibitor plus 49ml diluted buffer.

5. *Standard Plasma*
Add 1ml distilled water, leave for 5 minutes at room temperature and then mix gently until completely dissolved. Stable for 8 hours at 4°C.

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 2 hours of blood collection and should be assayed immediately or stored frozen at -20°C.

PREPARATION OF THE STANDARD CURVE

Dilute the Standard Plasma in buffer containing Kallikrein Inhibitor as follows:

STANDARD %	PLASMA (µl)	BUFFER (µl)
150	25	1475
100	25	1975
	From the 100% Standard Prepare:	
75	300	100
50	200	200
25	100	300
0	Use buffer plus Kallikrein Inhibitor alone	

Dilute 25µl of each test plasma with 1975µl of buffer plus Kallikrein Inhibitor.

ASSAY METHOD

Warm the substrate to 37°C. Into siliconised semimicro cuvettes or plastic tubes pipette:

Plasma dilutions or buffer plus Kallikrein Inhibitor	200µl

Incubate for 2 minutes at 37°C, add:

αFXIIa	200µl

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

Factor XII Substrate	200µl

Record the change in optical density at 405nm (rate assay), or incubate for exactly 30 minutes at 37°C, add:

Acetic Acid 50% (end-point assay)	200µl

Read the absorbance at 405nm (end-point assay).

CALCULATION

For the end-point assay, prepare blanks by substituting 400µl buffer for the αFXIIa and the Factor XII Substrate. Subtract the blank values from the test values. Plot the results as Log A_{405} against percentage αFXIIa Inhibitor 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:

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

*The potency of the standard plasma for αFXIIa Inhibitors (lot UD-0137-0466) is 1.10 U/ml.

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.

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