CHROMOGENIC SUBSTRATE ASSAY FOR DETERMINATION OF 
βFACTOR XIIa INHIBITORS

This kit is designed for the determination of 
β-Factor XIIa (βFXIIa) Inhibitors in human plasma. 
Purified βFXIIa is added to dilute plasma and a 
proportion of the enzyme complexes to its plasma 
inhibitors. The residual βFXIIa 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 
βFXIIa inhibitors.

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

1. βFactor XIIa
Dissolve in 10ml sterile distilled water. Stable at 
4°C for up to 6 hours, store 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 in aliquots 
below -20°C.

3. βFXIIai Buffer Concentrate
Dilute the vial contents (10ml) with 90ml distilled 
water. This gives a buffer of 0.05M Tris-HCl pH 7.9.

4. Kallikrein Inhibitor
Dissolve the contents of 1 vial in 10ml distilled 
water. Stable for 1 month at -20°C. Dilute 5ml of 
kallikrein inhibitor with 95ml buffer. Stable for 
8 hours at 4°C.

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:

<table>
<thead>
<tr>
<th>Standard (%) Activity</th>
<th>Plasma (μl)</th>
<th>Buffer (μl)</th>
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</thead>
<tbody>
<tr>
<td>150</td>
<td>25</td>
<td>1475</td>
</tr>
<tr>
<td>100</td>
<td>25</td>
<td>1975</td>
</tr>
</tbody>
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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 dilution or 
Buffer plus Kallikrein Inhibitor 200μl

Incubate for 4 minutes at 37°C, add:

βFXIIa 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 400ul buffer for the βFXIIa and the Factor XII Substrate. Subtract the blank values from the test values. Plot the results as Log A_m against percentage βFXIIa 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:

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

The potency of the standard plasma for βFXIIa Inhibitors (lot UD-0137-0466) is 1.25 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.

CATALOGUE NUMBER: 0052
PRODUCT: Unittest Beta FXIIai
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