INTENDED USE
For quantitative determination of Prothrombin (FII) functional activity in plasma and FII containing concentrates. The method is suitable for plasma collected in citrate or EDTA.

BIOCHEMISTRY
Factor II is a single chain vitamin K dependent glycoprotein of 72 kDa, which is activated to thrombin (FIIa) by FXa in the presence of FVa, calcium ions and phospholipids.

MEASUREMENT PRINCIPLE
FII functional activity is determined in a chromogenic prothrombinase method, in which human FII is activated to thrombin (FIIa) by human FXa in the presence of bovine FV, calcium ions and phospholipids. The amount of FIIa formed is determined from the hydrolysis of a chromogenic FIIa substrate. The FII activity of the sample is assigned vs. a normal human plasma calibrated against a WHO International Standard.

KIT COMPOSITION
Activator Reagent, 3.0 mL (4 vials) – REF 2010
The Activator Reagent contains lyophilized human FXa, bovine FVa, CaCl2 and phospholipids. Each vial is sufficient for 25 tests.

FII Diluent Buffer, Stock Solution, 20 mL (1 vial) – REF 2050
Liquid stock solution of diluent buffer.

Preparation of Factor II Standard Dilutions - Plasma

<table>
<thead>
<tr>
<th>Predilution</th>
<th>Volume of Predilution</th>
<th>Volume of Diluent Buffer working solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:50</td>
<td>20 µL</td>
<td>980 µL</td>
</tr>
<tr>
<td>1:133</td>
<td>300 µL of predilution</td>
<td>500 µL</td>
</tr>
<tr>
<td>1:160</td>
<td>200 µL of predilution</td>
<td>440 µL</td>
</tr>
<tr>
<td>1:200</td>
<td>100 µL of predilution</td>
<td>300 µL</td>
</tr>
<tr>
<td>1:300</td>
<td>100 µL of predilution</td>
<td>500 µL</td>
</tr>
<tr>
<td>1:600</td>
<td>50 µL of predilution</td>
<td>550 µL</td>
</tr>
<tr>
<td>1:2000</td>
<td>25 µL of predilution</td>
<td>975 µL</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>500 µL</td>
</tr>
</tbody>
</table>

NOTE: 100% activity is defined as a FII activity of 1 IU/mL in plasma. In case the FII activity of the plasma standard differs from this value, an appropriate correction factor should be used when calculating the sample result. It is recommended to express all sample results as IU/mL.

STORAGE AND STABILITY
The sealed reagents are stable at 2-8°C until the Expiry Date printed on the label. Be careful to avoid contamination of the reagents by microorganisms.

MATERIALS REQUIRED BUT NOT PROVIDED
- Deionized water, NCCLS Type II water or Ph Eur water for injection of higher quality.
- Human Plasma or FII concentrate, potency assigned vs. a WHO International Standard for FII activity.
- Citric acid, 2% (for end-point method)
- Calibrated pipettes
- Photometer, 405 nm (and 490 nm for end-point method)
- Heat incubator 37°C
- Plastic test tubes and Vortex mixer
- Stop-watch

SYMBOLS USED

REF
Catalog number

LOT
Batch code

Use by

Temperature limitation

Consult instruction for use

Biological risks

Manufacturer

PRECAUTIONS AND WARNINGS
CAUTION:
Each donor unit used in the preparation of the Activator Reagent has been tested by FDA approved methods for the presence of Hepatitis B surface antigen and antibodies to HIV 1 and 2 and Hepatitis C and found to be negative. However, since no test can completely rule out the presence of these blood borne diseases, the handling and disposal of this human source reagent should be made with care.
- Avoid contact with skin and eye.
- Do not empty into drains.
- Wear suitable protective clothing.

METHOD - Plasma
A calibration curve should be included in each run. A normal human plasma calibrated against a WHO International Standard should be used as calibrator. Prepare all dilutions in plastic tubes.

Preparation of standard dilutions - Plasma
Prepare independent predilutions for each standard dilution.

Sample dilution - Plasma
Plasma samples should be analyzed with a sample dilution of 1:200. The FII activity of the tested sample is derived from the calibration curve.

Sample blank
Due to the high sample dilution, a sample blank does not have to be included when analyzing plasma samples.
11 METHOD – CONCENTRATES

A calibration curve should be included in each run. A Factor II concentrate calibrated against a WHO International Standard should be used as calibrator. Prepare all dilutions in plastic tubes.

11.1 Preparation of standard dilutions - FII containing concentrates

Prepare independent predilutions for each standard dilution.

Example, using the 3rd International Standard for Factors II and X Concentrates 98/590 with an assigned Factor II activity of 11.2 IU/ampoule: Reconstitute one ampoule with 1.0 mL of distilled water followed by a predilution of 1:560 in Diluent Buffer-working solution to arrive at 20 mIU/mL. Prepare standard dilutions using the predilution of 20 mIU/mL according to the table below.

NOTE: The table below for preparation of standard dilutions is provided as an example only. Any dilution scheme resulting in final standard dilutions in the range 0.5 – 7.5 mIU/mL could be used.

<table>
<thead>
<tr>
<th>Predilution</th>
<th>Volume of Predilution</th>
<th>Volume of Diluent Buffer working solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mIU/mL</td>
<td>25 µL</td>
<td>975 µL</td>
</tr>
<tr>
<td>1.67 mIU/mL</td>
<td>50 µL</td>
<td>550 µL</td>
</tr>
<tr>
<td>3.33 mIU/mL</td>
<td>100 µL</td>
<td>500 µL</td>
</tr>
<tr>
<td>5 mIU/mL</td>
<td>100 µL</td>
<td>300 µL</td>
</tr>
<tr>
<td>6.25 mIU/mL</td>
<td>200 µL</td>
<td>440 µL</td>
</tr>
<tr>
<td>7.5 mIU/mL</td>
<td>300 µL</td>
<td>500 µL</td>
</tr>
</tbody>
</table>

11.2 Sample dilution - FII containing concentrates

Prepare sample dilutions in FII Diluent Buffer working solution to obtain activities in the range 0.5 – 7.5 mIU/mL. It is recommended to analyse FII concentrate samples at several different dilutions, starting at a FII activity of about 7.5 mIU/mL, to establish the minimal dilution required to avoid any matrix interference. All dilutions should be prepared in plastic tubes.

12 ASSAY – PLASMA AND CONCENTRATES

The same assay procedure should be used for both plasma and concentrates.

- **Sample / Standard dilution**: 50 µL
  - **Incubation 2-4 min, 37°C**
  - **Activator Reagent (37°C)**: 100 µL
  - **Activation 15 min, 37°C**
  - **FII Substrate (37°C)**: 50 µL
  - **Kinetic method**: Read ΔA405/min at 37°C
  - **End-point method**: Incubate at 37°C for 10 min
  - **Citric Acid, 2% (End-point method only)**: 50 µL

Kinetic reading:
- Read the absorbance change at 405 nm. End-point method: Stop the reaction with 2% citric acid after 10 min hydrolysis at 37°C. Read the absorbance at 405 nm, using 490 nm as reference wavelength. Absorbance readings should be made within 2 hours after termination of the substrate hydrolysis.

Express the sample result as IU/mL or %.

13 CALCULATION

Plasmas:
- Plot the maximal absorbance change/minute (ΔA405/min) or absorbance (A405-490) vs. FII activity in a Lin-Lin graph. Use a quadratic curve fit (Fig 1).
- The FII activity of the tested sample is obtained directly from the calibration curve. Correct the obtained value with an appropriate correction factor if the FII activity of the normal plasma standard differs from 1 IU/mL.
- If several sample dilutions are used, adjust for the dilution with the appropriate dilution factor.
- Express the sample result as IU/mL or %.

14 PERFORMANCE CHARACTERISTICS

The assay allows detection of about 0.25 mIU/mL (5%) FII activity.

15 EXPECTED VALUES

Normal Factor II levels in plasma range from 0.75 - 1.3 IU/mL.

16 INTERFERENCE

Factor II results are not affected at plasma levels up to the stated levels below:

- Hemoglobin: 10 mg/mL
- Bilirubin: 800 µg/mL
- Triglycerides: 10 mg/mL
- Heparin, LMW: 4 U/mL
- Heparin, UF: 4 U/mL

17 REFERENCES

1. 6th Edition of the European Pharmacopoeia, General Chapter 5.3 Statistical analysis of results of biological assays and tests.