BIOPHEN™ Factor VIIa

REF 221312

R1 R3 2 x 4 mL, R2 2 x 2 mL, R4 2 x 25 mL

Quantitative determination of Factor VIIa activity, in purified medium, using chromogenic assay.

FOR RESEARCH USE ONLY.
DO NOT USE IN DIAGNOSTIC PROCEDURES.

HYPHEN BioMed

Sales and Support:
CoaChrom Diagnostica GmbH
www.coachrom.com | info@coachrom.com
Tel: +43-1-236 222 1 | Fax: +43-1-236 222 111
Toll-free contact for Germany:
Tel: 0800-24 66 33-0 | Fax: 0800-24 66 33-3

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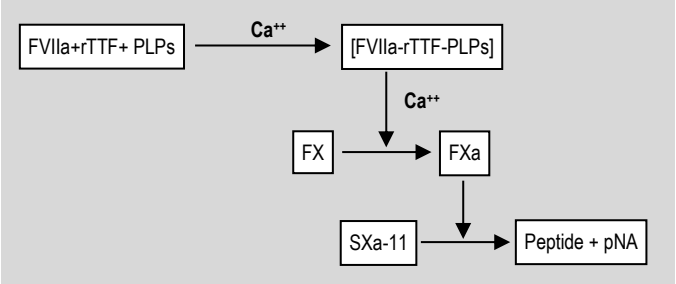
INTENDED USE:

The BIOPHEN™ Factor VIIa kit is a chromogenic method intended for *in vitro* quantitative determination of activated Factor VII (FVIIa) activity, in purified milieu, using manual or automated method.
This kit is for research use only and must not be used for patient diagnosis or treatment.

PRINCIPLE:

Factor VII is the serine esterase of the extrinsic coagulation pathway. When complexed to Tissue Factor (TF), in presence of phospholipids and calcium, it activates Factor X to Factor Xa.
The BIOPHEN™ Factor VIIa kit is insensitive to Factor VII.
Factor VIIa forms an enzymatic complex with recombinant truncated human Tissue Factor (rTTF) and synthetic phospholipids. It then activates Factor X, present in the assay at a constant concentration and in excess, to Factor Xa. The FXa activity is measured using a specific chromogenic substrate (SXa-11). Factor Xa cleaves the substrate and generates pNA. The amount of pNA generated is directly proportional to the Factor Xa activity. Finally, there is a direct relationship between the amount of Factor VIIa in the assayed sample and the Factor Xa activity generated, measured by the amount of pNA released, determined by colour development at 405 nm.



REAGENTS:

R1 **Human Factor X,** at the optimized concentration for the assay, lyophilised in presence of stabilizers. Contains BSA.
2 vials of 4 mL.
R2 **Cofactor (rTTF) and synthetic phospholipids,** at the optimized concentration for the assay, lyophilised in presence of stabilizers. Contains BSA.
2 vials of 2 mL.
R3 **Chromogenic substrate** specific for Factor Xa (SXa-11), lyophilized.
2 vials of 4 mL.
R4 **Specific Tris-BSA dilution buffer,** at pH 7.50. Ready to use. Contains BSA.
2 vials of 25 mL.

Reagent R4 contains small amounts of sodium azide (0.9 g/L), see WARNINGS AND PRECAUTIONS.

WARNINGS AND PRECAUTIONS:

- Biological products must be handled with all necessary precautions and considered as being potentially infectious.
- In contact with lead or copper pipes, sodium azide can generate explosive compounds.
- A yellow color indicates a contaminated substrate. Discard the vial and use a new one.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits. Do not mix reagents from different kit batches when performing an assay; they are optimized for each batch of kits.
- Handle the reagents with care to avoid contamination during use. If possible, avoid reagent evaporation during use by limiting the liquid-air exchange surface. Evaporation reduces the reagent's stability in the analyzer.
- To preserve reagent stability, seal the vials after use with their respective caps.
- Aging studies, conducted over a 3-week period at 30°C, show that the reagents can be shipped at room temperature over a short period of time, without degradation.
- The human plasma used to prepare the Factor X has been tested by recorded methods and is certified free of HIV antibodies, Hbs Antigen and HCV antibodies. The bovine plasma used to prepare the BSA has been tested by recorded methods and is certified free of infectious agents, in particular the causative agent of bovine spongiform encephalitis.
- Create a plasma blank if this latter is icteric, lipaemic, haemolysed, or if its color differs from the standard plasmas.
- When employing the kinetic method, use ΔOD 405 instead of OD 405.
- For *in vitro* use.

R1 R2 H315 : Causes skin irritation.
H319 : Causes serious eye irritation.
H335 : May cause respiratory irritation.

REAGENT PREPARATION AND STABILITY:

The reagents are lyophilized under a vacuum in their vials. To avoid any product loss when opening the vial of lyophilized reagents, gently remove the freeze-drying stopper.

R1 **Reagent 1**: **Human Factor X**
Reconstitute the contents of each vial with exactly **4 mL distilled water**, shake vigorously until fully dissolved. Allow to stabilize for 30 min. at room temperature (18-25°C), shaking occasionally.
Homogenize the reagent prior to use.
Reagent stability after reconstitution, excluding any contamination or evaporation, and stored in the original vial, is of:
- **72 hours** at 2-8°C.
- **24 hours** at room temperature (18-25°C).

R2 **Reagent 2**: **Cofactor (rTTF) and synthetic phospholipids**
Reconstitute the contents of each vial with exactly **2 mL distilled water**, shake vigorously until fully dissolved. Allow to stabilize for 30 min. at room temperature (18-25°C), shaking occasionally.
Homogenize the reagent prior to use.
Reagent stability after reconstitution, excluding any contamination or evaporation, and stored in the original vial, is of:
- **72 hours** at 2-8°C.
- **24 hours** at room temperature (18-25°C).

R3 **Reagent 3**: **Chromogenic substrate**
Reconstitute the contents of each vial with exactly **4 mL distilled water**, shake vigorously until fully dissolved. Allow to stabilize for 30 min. at room temperature (18-25°C), shaking occasionally.
Homogenize the reagent prior to use.
Reagent stability after reconstitution, excluding any contamination or evaporation, and stored in the original vial, is of:
- **1 month** at 2-8°C.
- **7 days** at room temperature (18-25°C).

R4 **Reagent 4**: **Special Tris-BSA dilution buffer**
Ready to use. Allow to stabilize for 30 minutes at room temperature (18-25°C), before use.
Homogenize the reagent prior to use.
Reagent stability after opening, excluding any contamination or evaporation, and stored in the original vial, is of:
- **7 days** at 2-8°C.
- **7 days** at room temperature (18-25°C).

STORAGE CONDITIONS:

Unopened reagents should be stored at 2-8°C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:
- Distilled water.
- 20% acetic acid or 2% citric acid (end point method).
- Specific calibrators and controls with known concentrations such as International Standard for FVIIa NIBSC[1] or internal reference preparations and controls for FVIIa.

Materials:
- Spectrophotometer or automatic instrument for chromogenic assays.
- Stopwatch, Calibrated pipettes.

PROCEDURE:

The kit can be used for kinetics, automated or manual (endpoint) methods. Perform the test at **37°C** and read color intensity at **405nm**.

Automated methods:
Applications for various analyzers are available on request. **See the specific application and specific precautions for each analyzer.**

Assay method (manual):

1. Reconstitute the reference preparation or calibrator and controls (two levels recommended at about 75 and 250 mIU/mL) using the specific package inserts or according to internal procedure.
The calibration curve can also be established from a Factor VIIa titrated reference material (international standard or internal standard).
Pre-dilute this material in **R4** buffer (**at least 1:2 or more**) to obtain approximately a **400 mIU/mL** solution (noted C), and then use this solution to establish a calibration curve in R4 buffer.
Prepare calibration points in the range about 0-400 mIU/mL (see example table below).

Calibrator	C	C/2	C/4	C/8	C/12	0
FVIIa (mIU/mL)	~400	~200	~100	~50	~33,3	0
Volume calibrator	1 mL	0,5 mL	0,25 mL	0,125 mL	0,0833 mL	0 mL
Volume buffer R4	0 mL	0,5 mL	0,75 mL	0,875 mL	0,9167 mL	1 mL

Additional low calibration points can be added if required. In order to get the full assay performances, the calibration curve must be prepared just before running the assay.

For information, correspondence between ng/mL and IU/mL:

Concentration in ng/mL	Concentration in IU/mL
20 ng/mL	1 IU/mL
1 ng/mL	50 mIU/mL

2. Dilute the sample and controls in R4 buffer, as described in the table below:

Samples	Predilution
Samples (FVIIa in purified medium)	Adjusted to 50-350 mIU/mL optimal range in R4 buffer (1:2 or more)
Controls	1:2 or more

Establish the calibration curve and test it with the quality controls.

For FVIIa in purified medium, the tested specimen can be pre-diluted in **R4** Buffer (**at least 1:2 or more**), to obtain an expected FVIIa concentration in the range **0 to C mIU/mL** (the measured concentration must then be multiplied by the "pre-dilution" factor).

3. Dispense the following to the wells of a microplate, or to a plastic tube incubated at **37°C**:

Reagents	Microplate	Volume
Calibrators, Controls or tested specimen (**prediluted in R4**)	30 µL	100 µL
R2 : Cofactor-PLPs preincubated **at 37°C**	30 µL	100 µL
R1 : Factor X preincubated **at 37°C**	60 µL	200 µL
Mix and Incubate for **exactly 4 min at 37°C**, then introduce:		
R3: SXa-11 Substrate preincubated **at 37°C**	60 µL	200 µL
Mix and Incubate for **exactly 4 min at 37°C**, exactly		
Stop the reaction by adding:		
Citric Acid (2%)*	60 µL	200 µL
Mix and measure the optical density at **405nm** against the corresponding blank.		

*Or acetic acid (20%). The yellow color is stable for 2 hours.
The specimen blank is obtained by mixing the reagents in the reverse order to that of the test: Citric acid (2%), R3, R1, R2, dilute specimen**.**
Measure the optical density at **405 nm**. Subtract the measured blank value from the absorbance measured for the corresponding test.

Assay method (kinetics)

The assay can be read using a kinetics mode. In this case the change in absorbance is recorded between two points following the addition of substrate. There is then no need to subtract the sample blank, or to stop the reaction. The results are obtained using the change in absorbance (ΔOD405) for calibrators, controls and tested specimen.

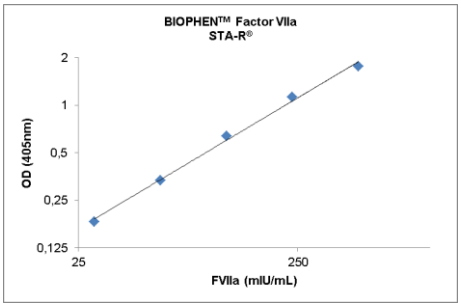
If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

CALIBRATION:

The BIOPHEN™ Factor VIIa assay can be calibrated for the assay of Factor VIIa activity in purified medium.
Using a logarithmic scale:

- The calibration is linear from about 0 to 400 mIU/mL.

The calibration curve shown below, obtained with purified recombinant FVIIa, on STA-R® analyzer, is given by way of example only. The calibration curve established for the assay series must be used.



QUALITY CONTROL:

The use of quality controls serves to validate method compliance, along with between-test assay homogeneity for a given batch of reagents.
Include the quality controls with each series, as per good laboratory practice, in order to validate the test. A new calibration curve should be defined, preferably for each test series, and at least for each new reagent batch, or after analyzer maintenance, or when the measured quality control values fall outside the acceptable range for the method.
Each laboratory must define its acceptable ranges and verify the expected performance in its analytical system.

RESULTS:

- For the manual endpoint method, plot the calibration curve (Log-Log), with the OD 405 nm along the Y-axis and the FVIIa concentration, expressed as mIU/mL, along the X-axis.
- The concentration of FVIIa in the diluted test sample, is deduced from the calibration curve. When predilution is used, multiply the measured FVIIa concentration by the predilution factor in order to get the concentration in the tested sample (e.g. multiplied by 2 when 1:2 dilution is used).
- Results are expressed in mIU/mL.

The results obtained should be for research use only and must not be used for patient diagnosis or treatment.

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully. The laboratory is responsible for validating any changes made to these instructions for use.
- Any reagent presenting an unusual appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- For samples measured > 400 mIU/mL, an additional 2 fold (or more) dilution can be used and obtained results multiplied by the additional dilution factor.

PERFORMANCE:

- The detection threshold for the assay is evaluated on the calibration curve by measuring the "apparent" FVIIa concentration, which corresponds to the mean OD at 405 nm value obtained for a sample free of FVIIa plus 3 Standard Deviations (SD). This **detection threshold** is below **25 mIU/mL FVIIa**.
- The assay working range is from about 25 to 400 mIU/mL.
- **Specificity**: The assay is specific for FVIIa, FVII is not measured (as an indication, reactivity of FVII is expected being <1% the one obtained for FVIIa).
- Inter assay CV is expected <10%.

REFERENCES:

1. WHO International Standard, Blood Coagulation Factor VIIa, concentrate, Human, 2nd International Standard, NIBSC, 07/228.

SYMBOLS:

Symbols used and signs listed in the ISO 15223-1 standard, see Symbol definitions document.

Changes compared to the previous version.