BIOPHEN™ FVII

REF 221304

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

Chromogenic assay for quantitative determination of Factor VII

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES

HYPHEN BioMed
www.hyphen-biomed.com
155, rue d'Eragny
95000 NEUVILLE SUR OISE
FRANCE
Tel. : +33 (0)1 34 40 65 10
Fax : +33 (0)1 34 48 72 36
info@hyphen-biomed.com

English, last revision: 03-2020

INTENDED USE:

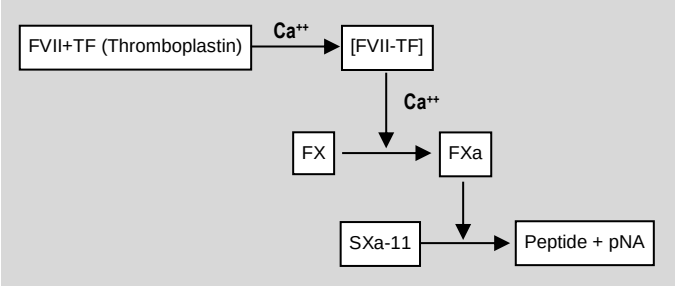
The BIOPHEN™ FVII kit is a chromogenic method for quantitative determination of the Factor VII (FVII) activity in purified medium or citrated plasma using a chromogenic method, manual or automated.
This kit is for research use only and should not be used for patient diagnosis or treatment.

SUMMARY AND EXPLANATION:

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 (FX) to Factor Xa (FXa).
BIOPHEN™ FVII kit is a chromogenic assay for testing Factor VII activity.

ASSAY PRINCIPLE:

In the presence of tissue factor (thromboplastin) and calcium, FVII forms an enzymatic complex which activates FX, present at a constant concentration and in excess, to FXa. The amount of formed FXa depends on the concentration of FVII to be assayed. This formed FXa cleaves the specific FXa substrate (SXa-11) and releases pNA. The amount of pNA generated is directly proportional to FXa activity.
The amount of FVII in the assayed sample is directly proportional to the FXa activity generated, measured by the amount of pNA released, determined by color development at 405 nm.



REAGENTS:

R1 Factor X (Human), lyophilized. Contains Factor X at the optimized concentration for the assay and BSA.
2 vials of 4mL.
R2 Thromboplastin Calcium, lyophilized. Contains thromboplastin, calcium and BSA.
2 vials of 2mL.
R3 SXa-11, lyophilized. Chromogenic substrate, specific for Factor Xa (SXa-11). Contains 8 mg of SXa-11.
2 vials of 4mL.
R4 Tris-BSA buffer at pH7.40, ready to use. Contains BSA.
4 vials of 25 mL.

Reagent R4 contains low concentration of Sodium azide (0.9 g/L), see CAUTIONS AND WARNINGS

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of human and animal origin. Whenever human plasma is required for the preparation of these reagents, approved methods are used to test the plasma for the antibodies to HIV 1, HIV 2 and HCV, and for hepatitis B surface antigen, and results are found to be negative. However, no test method can offer complete assurance that infectious agents are absent. Therefore, users of reagents of these types must exercise extreme care in full compliance with safety precautions in the manipulation of these biological materials as if they were infectious.
- Sodium azide can generate explosive components in contact with lead or copper pipes.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.
- Aging studies show that the reagents can be shipped at room temperature without degradation.
- This device of *in vitro* use is intended for professional use in the laboratory.

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

REAGENT PREPARATION:

Gently remove the freeze-drying stopper, to avoid any product loss when opening the vial.

Reconstitute the contents of each vial with exactly :
R1 R3 4 mL of distilled water.
R2 2 mL of distilled water.
Shake vigorously until complete dissolution while avoiding formation of foam and load it directly on the analyzer following application guide instruction.
For manual method, allow to stabilize for 30 minutes at room temperature (18-25°C), homogenize before use.

R4 Reagent is ready to use. Homogenize the reagent before each use.

STORAGE AND STABILITY:

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.

R1 R2 Reagent stability after reconstitution, free from any contamination or evaporation, and stored closed, is of:

- **48 hours** at 2-8°C.
- **8 hours** at room temperature (18-25°C).
- **Do not freeze.**
- **Stability on board of the analyzer: see the specific application.**

R3 Reagent stability after reconstitution, free from any contamination or evaporation, and stored closed, is of:

- **3 months** at 2-8°C.
- **7 days** at room temperature (18-25°C).
- **Do not freeze.**
- **Stability on board of the analyzer: see the specific application.**

If the substrate becomes yellow, this indicates a contamination. Discard the vial and use a new one.

R4 The reagent, provided that any contamination or evaporation is avoided, kept in its original vial is stable until the expiration date printed on the label when stored at 2-8°C.

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- 20% acetic acid or 2% citric acid (end point method).
- Specific Calibrators and controls with a known concentration such as:

Product Name	Reference
BIOPHEN™ Plasma Calibrator	222101-RUO
BIOPHEN™ Normal Control Plasma	223201-RUO
BIOPHEN™ Abnormal Control Plasma	223301-RUO

Also refer to the specific application guide of the analyzer used.

Materials:

- Spectrophotometer or automatic instrument for chromogenic assays.
- Stopwatch; Calibrated pipettes; Plastic tubes or microplate.

SPECIMEN COLLECTION AND PREPARATION:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture. Discard the first tube.
Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5[7] guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references[8].

TEST PROCEDURE:

The BIOPHEN™ FVII kit can be used for kinetics methods, automated on instruments, or by manual method (end point). The assay is performed **at 37°C** and the color intensity is measured **at 405 nm**.

For an automated method, application guides are available on request. See specific application guide and specific precautions for each analyzer.

Assay method:

1. For the plasmatic medium, calibration is performed with a plasma calibrator with a concentration **(C)** in FVII precisely defined or a normal pooled citrated plasma (at least 30 normal individuals, males or females, aged between 18 and 55 years, and free of any medication or disease), with the assigned value of **100 %** FVII. The assay includes a standard plasma dilution of **1:1000**. By definition, this latter dilution of the pool represents the **100%** FVII activity. The dynamic range is from **0 to 200%** FVII. The **200%** FVII activity is the **1:500** dilution of the **plasma pool** or **calibrator**.
The **1:1000** dilution corresponds to the indicated FVII concentration **(C)**, and the **1:500** to twice this concentration. For a calibrator titrating **C**, the **200%** FVII concentration is obtained (in the assay conditions) by using the following dilution factor: **500xC:100.**
In order to have an accurate dilution, predilute the pool plasma at **1:25**, then **1:20** with Tris-BSA buffer **R4** to obtain a **1:500 final dilution** (i.e. **200%** FVII). Using this dilution, prepare the calibration range as indicated here below:

FVII (%)	0	50	100	200
Plasma calibrator diluted 1:500 (µL)	0	125	250	500
R4: Tris-BSA buffer (µL)	500	375	250	0

2. Dilute the samples using Tris-BSA buffer **R4** as described in the table below:

Sample	Reference	Dilution
Controls	223201-RUO 223301-RUO	**1:1000**
Specimen	n.a.	**1:1000**

Establish the calibration curve and test it with the quality controls. When stored at room temperature (18-25°C), test the diluted specimens quickly. The exact calibrator and control concentrations for each batch are indicated on the flyer provided with the kit.

3. For purified medium with FVII concentrations different from that of plasma, dilute the sample in **R4** in order to get a final FVII concentration in the tested dilution in the range **0.1 to 1ng/mL** (i.e. **20 to 200%** FVII, using this protocol).

4. In a plastic tube or in microassay well, incubated at **37°C**, introduce:

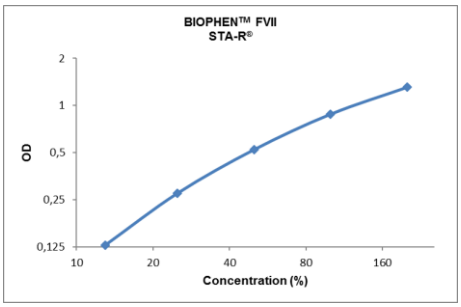
	Microplate	Test tube
Calibrators, Controls, or tested plasmas **diluted**	30µL	100µL
R2 Thromboplastin Calcium preincubated at **37°C**	30µL	100µL
R1 Factor X (human) preincubated at **37°C**	60µL	200µL
Mix and incubate at 37°C, for **7 minutes**, then introduce:		
R3 SXa-11 preincubated at **37°C**	60µL	200µL
Mix and incubate at 37°C for **exactly 5 minutes:**		
Stop the reaction by introducing:		
Citric acid (2%)*	60µL	200µL
Mix and measure the absorbance at **405nm** against the corresponding blank.		

*Or acetic acid (20%). The yellow color is stable for 2 hours.
The sample blank is obtained by mixing the reagents in the reverse order from that of the test i.e.: Citric acid (2%)**,** R3, R1, R2, diluted plasma**.**
Measure the absorbance **at 405 nm**. The sample blank value must be deduced from the absorbance measured for the corresponding assay.

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™ FVII assay can be calibrated for measurement of Factor VII. Calibrator which covers the test dynamic range is available at HYPHEN BioMed (see table in the REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED section) and can be used to generate calibration curves.
The calibration curve below, obtained with the calibrator BIOPHEN™ Plasma Calibrator on STA-R® is indicated as an example only. The calibration curve generated for the series of measures performed must be used.



QUALITY CONTROL:

Using quality controls allows validating the calibration curve, as well as the homogeneous reactivity from run to run, when using a same lot of reagents. Quality control must be included in each series, as per good laboratory practice, in order to validate test results. A new calibration curve must be carried out preferentially for each test series, and at least for each new lot of reagents or, after each important analyzer's maintenance, or when quality controls values are measured outside the acceptance range determined for the method.
Each laboratory has to establish and verify its own target values, acceptance ranges and expected performances, according to the instruments and protocols used.

RESULTS:

- For the end point manual method, plot the calibration curve log-log, with the absorbance OD at 405 nm along the Y-axis and the FVII concentration, expressed as %, along the X-axis. The plot must correspond to a polynomial of degree 2.
- The FVII concentration in the tested specimen is directly deduced from the calibration curve.
- Results are expressed as % of Factor VII.
- When the assay dilution is **1:1000**, the Factor VII concentration is directly read on the calibration curve. When different dilutions are used, the rate measured must be multiplied by the dilution factor "**D**", divided by **1000**, i.e. **D/1000**.

The results obtained should be for research purposes only and not 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.
- Any reagent presenting an unusual aspect or contamination signs must be rejected.
- Any plasma containing a coagulum or contamination signs must be rejected.

PERFORMANCES:

- The lower limit of detection is ≤ 5%.
- The assay working range is from 5 to 200%.

REFERENCES:

1. Seligsohn U, *et al,*. Coupled amidolytic assay for factor VII: its use with a clotting assay to determine the activity state of factor VII Blood. 1978.
2. Clarke BJ, *et al*,. The first epidermal growth factor domain of human coagulation factor VII is essential for binding with tissue factor. FEBS. 1992.
3. Ledwozyw A, *et al*,. The estimation of factor VII in livestock plasma of domestic animals by the use of tripeptide chromogenic substrate. Arch Vet Pol. 1993.
4. Chang YJ, *et al*,. Engineered recombinant factor VII Q217 variants with altered inhibitor specificities. Biochemistry. 1999.
5. Natacha CJ, *et al*,. Increased volume of distribution for recombinant activated factor VII and longer plasma-derived factor VII half-life may explain their long lasting prophylactic effect. Thrombosis Research. 2013.
6. Dorkin JR. Development and mechanistic analysis of in vivo liposomal nanoparticle delivery of siRNA and mRNA. B.A. Biological Chemistry Swarthmore College. 2006.
7. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008
8. Woodhams B. *et al*. Stability of coagulation proteins in frozen plasma. Blood coagulation and Fibrinolysis. 2001.

SYMBOLS:

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

Changes compared to the previous version.