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

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INTENDED USE:

The BIOPHEN FVII kit is a chromogenic method for quantitative determination of the factor VII 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:

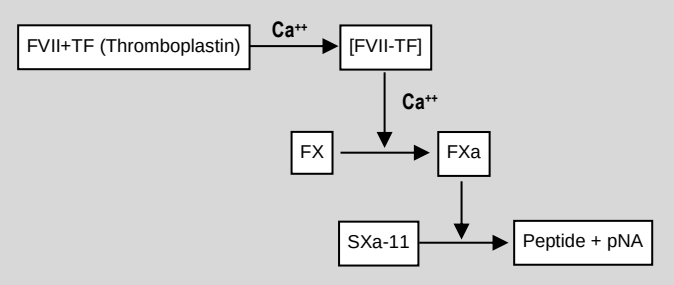
Factor VII is the serine esterase of the extrinsic coagulation pathway. When complexed to Tissue Factor (TF), in presence of phospholipids and Calcium, it activates Factor X to Factor Xa.

BIOPHEN FVII kit is a chromogenic assay for testing Factor VII activity.

ASSAY PRINCIPLE:

In the presence of tissue factor (rabbit thromboplastin) and calcium, factor FVII forms an enzymatic complex which activates the factor X, present at a constant concentration and in excess, to factor Xa. The amount of factor Xa formed depends on the concentration of Factor VII to be assayed. This factor Xa formed cleaves the specific substrate Xa (SXa-11) and releases pNA. The amount of pNA generated is directly proportional to the Factor Xa activity.

The amount of Factor VII in the assayed sample is directly proportional to the Factor Xa activity generated, measured by the amount of pNA released, determined by color development at 405 nm.



REAGENTS:

R1: Reagent 1: **Factor X (Human)**, lyophilized. Contains Factor X at the optimized concentration for the assay. 2 vials of 4mL.

R2: Reagent 2: **Thromboplastin Calcium**, lyophilized. Contains rabbit brain thromboplastin and calcium. 2 vials of 2mL.

R3: Reagent 3: **SXa-11**, lyophilized. Chromogenic substrate, specific for Factor Xa (SXa-11). Contains 8 mg of SXa-11. 2 vials of 4mL.

R4: Reagent 4: **Tris-BSA buffer** at pH7.40, ready to use. 4 vials of 25mL.

Reagent 4 contains low concentration of Sodium azide (0.9 g/L), see CAUTIONS AND WARNINGS

CAUTIONS AND WARNINGS:

- Any product of biological origin must be handled carefully, as being potentially infectious.
- Sodium azide can generate explosive components in contact with lead or copper pipes.
- If the substrate becomes yellow, this indicates the presence of a contaminant. It must be rejected, and a new vial must be used.
- The disposal of waste materials must be carried out according to current local regulations
- Use only reagents from kits with the same lot number. Do not mix reagents from kits with different lots when running the assay; they are optimized for each lot of kits.
- Reagents must be handled with care, in order to avoid any contamination during use. Take care to limit as much as possible any evaporation of the reagents during use, by limiting the liquid-air surface exchange. Evaporation reduces reagent stability on instrument board.
- In order to preserve the stability of the reagents, close the vials with their original screw cap following each use.
- Stability studies for 3 weeks at 30°C show that the reagents can be shipped at room temperature for a short period without damage.
- For in vitro use.

PREPARATION AND STABILITY OF REAGENTS:

Vials are closed under vacuum. Remove carefully the stopper, in order to avoid any loss of powder when opening the vials.

R1: **Reagent 1: Factor X (Human)**

Reconstitute each vial with **exactly 4 mL** of distilled water, shake thoroughly for complete dissolution. Let the reagent stabilize for 30 min at room temperature (18-25°C); while shaking from time to time.

Homogenize the reagent before each use.

Stability of reconstituted reagent, provided that any contamination or evaporation is avoided, kept in its original vial is:

- **48 hours** at 2-8°C.
- **8 hours** at room temperature (18-25 °C).
- **Do not freeze.**

R2: **Reagent 2: Thromboplastin Calcium**

Reconstitute each vial with **exactly 2 mL** of distilled water, shake thoroughly for complete dissolution. Let the reagent stabilize for 30 min at room temperature (18-25°C); while shaking from time to time.

Homogenize the reagent before each use.

Stability of reconstituted reagent, provided that any contamination or evaporation is avoided, kept in its original vial is:

- **48 hours** at 2-8°C.
- **8 hours** at room temperature (18-25 °C).
- **Do not freeze.**

R3: **Reagent 3: SXa-11 (Factor Xa specific chromogenic substrate)**

Reconstitute each vial with **exactly 4 mL** of distilled water, shake thoroughly for complete dissolution. Let the reagent stabilize for 30 min at room temperature (18-25°C); while shaking from time to time.

Homogenize the reagent before each use.

Stability of reconstituted reagent, provided that any contamination or evaporation is avoided, kept in its original vial is:

- **3 months** at 2-8°C.
- **7 days** at room temperature (18-25 °C).
- **Do not freeze.**

R4: **Reagent 4: Tris-BSA buffer**

Ready to use. Homogenize the reagent before each use.

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.

STORAGE CONDITIONS:

Unopened reagents must be stored at 2-8°C, in their original packaging box. They are then usable until the expiration date printed on the label.

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

Materials:

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

SPECIMEN COLLECTION:

Preparation and storage of specimens must be performed according to the current local regulations.

- Specimens:

Human plasma obtained from anticoagulated blood (trisodium citrate).

- Collection:

Blood (9 vol.) must be collected on trisodium citrate anticoagulant (1 vol.) (0.109M), with caution, through a net venipuncture. The first tube must be discarded.

- Centrifugation:

Within 2 hours, use a validated method in the laboratory to obtain a platelet-poor plasma, e.g., a minimum of 15 minutes at 2500 g at room temperature (18-25°C) and plasma must be decanted into a plastic tube.

- Storage of plasma:
  - 4 hours at room temperature (18-25°C)
  - 1 month at -20°C.
  - 18 months at -70°C.

Frozen plasma specimens should be rapidly thawed at 37°C, then gently mixed and tested immediately. Resuspend any precipitation by thorough mixing immediately after thawing and before testing.

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**.

Automated methods:

Applications to the various analyzers are available upon request. **Refer to each specific application and specific cautions for each analyzer.**

Assay method:

1. For the plasmatic medium, calibration is performed with a plasma calibrator with a concentration **(C)** in Factor VII 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 %** Factor VII. The assay includes a standard plasma dilution of **1:1000**. By definition, this latter dilution of the pool represents the **100%** Factor VII activity. The dynamic range is from **0 to 200%** Factor VII. The **200%** Factor VII activity is the **1:500** dilution of the **plasma pool** or **calibrator**.
The **1:1000** dilution corresponds to the indicated Factor VII 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: **1:500.**
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%** factor VII). 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**

Run the calibration curve and test it with quality controls. Diluted sample have to be tested within 2 hours when stored at room temperature (18-25°C).

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

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

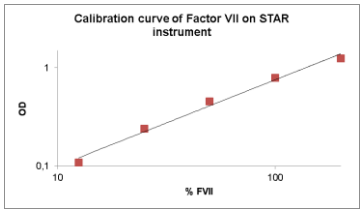
	Microplate	Test tube
Calibrators, Controls, or tested plasmas diluted **1:1000**	30µL	100µL
R2: Thromboplastin Calcium preincubated at **37°C**	30µL	100µL
R1: Factor X (human) preincubated at **37°C**	60µL	200µL
Mix and incubate at 37°C, for **7 minutes**, then introduce:		
R3: SXa-11 preincubated at **37°C**	60µL	100µL
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 another reactive volume than the one indicated here above is required for the method used, the volumes ratio must be strictly respected, in order to assure the assay performances. It is responsibility of the user to validate any modifications and their impact on all assay 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, draw the calibration curve on a logarithmic graph paper plot, with on abscissae, the Factor VII concentration (%) and on ordinates the corresponding absorbance OD_{450} (nm).
- 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:

- In order to get the optimal performances of the assay, the technical instructions must be strictly followed.
- 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:

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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. Woodhams B. *et al*. Stability of coagulation proteins in frozen plasma. Blood coagulation and Fibrinolysis. 2001.

SYMBOLS:

Used symbols and signs listed in the ISO standard 15223-1.