BIOPHEN Factor X
Ref 221705

Chromogenic assay for the measurement of Factor X in plasma

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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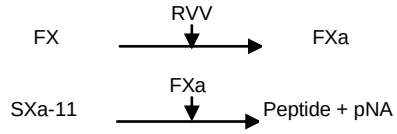
INTENDED USE:

BIOPHEN Factor X kit is an in vitro assay for the quantitative determination of Factor X in human citrated plasma with a chromogenic method, using manual or automated protocols.
This kit is for research use only and should not be used for patient diagnosis or treatment.

ASSAY PRINCIPLE:

Using the BIOPHEN Factor X assay, Factor X is measured following a specific activation with RVV, an enzyme extracted from snake venom (Russell's viper venom). Activated Factor X (FXa) then specifically cleaves the specific substrate SXa-11, releasing para-nitroaniline (pNA), which color is measured at 405nm. There is a direct relationship between color development and Factor X activity in the tested plasma.



REAGENTS:

R1: ***Reagent 1:*** **SXa-11 substrate**
Chromogenic substrate, specific for Factor Xa (SXa-11), lyophilised:
4 vials containing about 5mg of SXa-11 (to be reconstituted with **2.5 mL** of distilled water).

R2: ***Reagent 2:*** **RVV.**
Highly purified enzyme, extracted from the Russell's viper venom, lyophilized in the presence of calcium and stabilized; RVV in presence of calcium can specifically activate Factor X into Factor Xa.
4 vials (to be reconstituted with **2.5 mL** of distilled water).

R3: ***Reagent 3:*** **Tris-NaCl buffer "10xconc."**
Ten fold concentrated Tris-NaCl buffer. Contains sodium azide. To be diluted **ten fold** with distilled water before use. 4 vials containing about 5 ml.

Precaution and warnings:

- Bovine serum albumin (BSA) was prepared from bovine plasma, which was tested for the absence of infectious agents, and collected from animals free from BSE. However, no test may totally exclude the absence of infectious agents. As any product of bovine origin, reagents must be used with all the cautions required for handling a material potentially infectious.
- All the required cautions must be respected in order to avoid any risk of ingestion or accidental introduction of R1 or R2 in body. In case of skin contact, wash extensively with water. In case of contact with a wound, address to the appropriate medical service, and indicate the biological origin and the nature of the product.
- The buffer (R3) contains sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Flush with large volumes of water when discarding into a sink.
- Avoid contact with skin and eyes. Do not empty into drains. Wear suitable protective clothing.
- For in vitro research use.
- The RVV concentration may present variations from lot to lot, but it is exactly adjusted for each new lot of reagent, in order to allow obtaining a homogeneous reactivity.

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water, preferentially sterile.
- Acetic Acid (20%) or Citric Acid (2%) (End point method).
- Calibration and quality control plasmas, titrated for Factor X (eg Biophen Plasma Calibrator #222101; Biophen Normal Control Plasma #223201; Biophen Abnormal Control Plasma #223301).

Material:

- Spectrophotometer, photometer or automates for chromogenic assays, with a wave-length set up at 405 nm.
- Manual method: Water bath or incubator at 37°C, plastic tubes or microplates, stop watch.
- Calibrated pipettes.

STORAGE CONDITIONS:

BIOPHEN Factor X kits must be stored at 2-8°C, in their original packaging box. They are then stable until the expiration date printed on the box.

PREPARATION AND STABILITY OF REAGENTS:

R1: Reagent 1: Factor Xa specific Chromogenic substrate (SXa-11)
Reconstitute each vial with **2.5 ml** of distilled water; homogenize the contents. Incubate at Room Temperature (18-25°C) for 30 min, before use, while shaking from time to time.
Homogenize before use.

Stability of restored substrate, kept in its original vial:

- 1 month at 2-8°C.
- 3 days at Room Temperature (18-25°C).
- 1 month at -20°C (before use, thaw for 15 min. in a water bath at 37°C).

R2: Reagent 2: RVV
Reconstitute each vial with exactly **2.5 ml** of distilled water; homogenize the content. Let the reagent to stabilise for 30 min at Room Temperature, before use, while shaking from time to time.
Homogenize before use.

Stability of reconstituted RVV reagent, kept in its original vial:

- 1 week at 2-8°C.
- 3 days at Room Temperature (18-25°C).
- 1 month at -20°C (before use, thaw for 15 min. in a water bath at 37°C).

R3: Reagent 3: Tris-NaCl buffer "10xconc."
Ten fold concentrated Tris-NaCl buffer. Shake the vial and dilute the amount required **1:10** in distilled water (the 5 ml contained in the vial allow preparing 50ml of ready to use buffer). The buffer must be stored at 2-8°C in its original vial and used within 4 weeks following opening. The diluted buffer must be used within 7 days, when protected from any contamination and stored at 2-8°C.

Cautions:

- In order to improve stability, reagents must be closed with their original screw cap following each use (yellow caps for SXa-11, white caps for RVV, white cap for the buffer).
- Reagents must be handled with care, in order to avoid any contamination during use.
- If the substrate becomes yellow, this indicates the presence of a contaminant. It must be rejected, and a new vial must be used.
- To incubate the reconstituted vials, for 30 min. at RT, allows stabilising the reagents, and obtaining a homogeneous reactivity over time.
- In order to avoid evaporation of reagents, limit the exchange surface by using, for example, a vial neck or an operculated cap.

Note:

- R1 and R2 vials are closed under vacuum. Remove carefully the stopper, in order to avoid any lost of powder when opening the vials.
- According to the automated method used, the reagents can be reconstituted with volumes different from those recommended. In any case, the established reactive ratios (respective reagent concentrations in the reactive milieu) between R1 and R2 must be strictly respected.
- Use only reagents from kits with a same lot number. Do not mix reagents from kits with different lots when running the assay. Reagents R1 and R2 are optimized for each lot of kits.
- The stability studies at 30°C show that the reagents can be shipped at room temperature without damage.

PREPARATION OF PLASMA (SPECIMEN COLLECTION):

Blood (9 volumes) must be collected on 0.109 M citrate anticoagulant (1 volume), with great care, in a silicon glass or a plastic tube. Sampling must be performed through a net venipuncture, avoiding any blood activation.

- Within 4 hours, blood must be centrifuged at 3,000 g for 20 min at 18°C or below, and plasma decanted into a plastic tube, using a plastic pipette. Separate carefully plasma from blood cells.
- Storage of plasma:
  - Up to 8 hours at Room Temperature (18-25°C).
  - Up to 24 hours at 2-8°C.
  - Up to 1 month frozen at –20°C or below (before use, thaw for 15 min. in a water bath at 37°C).

Refer to NCCLS document H21-A2 for further instructions on specimen collection, handling and storage.

TEST PROCEDURE:

BIOPHEN Factor X kit is designed for being used with kinetics methods, automated, but it can also be used for end point manual methods. Adaptations for the various automates are available upon request. The assay is performed at the controlled temperature of 37°C and the color development is measured at 405 nm.

CALIBRATION:

Calibration is performed with a normal pooled citrated plasma (made with plasmas from 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 X. The assay includes a standard plasma dilution of **1:10**. By definition, this latter dilution of the pool represents the **100 %** Factor X activity. The dynamic range is from **0 to 200 %** Factor X. The **200 %** Factor X activity is then the **1:5** dilution of the **plasma pool** (in the ten fold diluted Tris-NaCl buffer (R3)).
(**Or** calibration is performed with a commercially available plasma calibrator, with a known Factor X concentration (**C**). The **1:10** dilution corresponds to the indicated Factor X concentration, and the **1:5** to twice this concentration. Using a plasma calibrator with a Factor X concentration of **C**, the **200%** Factor X concentration is obtained (in the assay conditions) by using the following dilution factor:
5 x C:100.)
The calibration curve can then be prepared as follows from the preparation already adjusted at 200% Factor X:

% FX	200%FX Calibrator (µl)	Diluted buffer (R3) (µl)
0	0	500
50	125	375
100	250	250
200	500	0

ASSAY PROTOCOL:

Manual Method:
Dilute the tested samples, and the controls **1:10** with Tris-NaCl buffer (**R3**, prediluted 1:10 in distilled water).
In a microplate well, or in a **plastic** tube preincubated at **37°C**, introduce:

Reagents	Microplate	Test Tube
Calibrators; Controls or tested plasmas **diluted 1:10**	50 µL	200 µL
Incubate for **1 to 2** min. at 37°C, then introduce:		
R1: SXa-11 Substrate preincubated **at 37°C**	50 µL	200 µL
Mix and incubate for **1 to 2** min. at 37°C, then introduce:		
R2 : RVV, preincubated **at 37°C**	50 µL	200 µL
Mix and Incubate for **2** min at 37°C, **exactly**		
Stop the reaction by introducing:		
Citric Acid (20g/L)	50 µL	200 µL
Mix and measure the optical density at **405 nm** against the sample blank.		

The yellow color obtained is stable for 2 hours.

The sample blank is obtained by mixing the reagents in the opposite order from that of the test i.e., Citric Acid (20 g/L), RVV, SXa-11 substrate, diluted plasma.
Measure the Absorbance at 405 nm (A405). Subtract the sample blank from the A405 obtained for the assay.

Automated methods:
Detailed instrument settings including instructions for the preparation of the reagents for a variety of automated instruments are available upon request.

Note:
- If higher or lower reactive volumes are required for the method used, the same respective proportions for each reagent concentration, and for the overall reactive volume, must be strictly respected, in order to keep the assay performances.
- Do a sample blank in presence of highly lipemic, icteric or hemolysed plasmas, or if plasma has a "color" different from the usual one**.**

QUALITY CONTROL:

Using commercially available quality control plasmas, titrated for Factor X, allows validating the calibration curve, as well as the homogeneous reactivity from run to run, when using a same lot of reagents. The calibration curve is acceptable when the concentrations measured for controls are within the acceptance range.
Various control plasmas are available: BIOPHEN Normal Control Plasma (#223201) and BIOPHEN Abnormal Control Plasma (#223301). Each laboratory should verify its own target value and acceptance range, in the exact working conditions, for each new lot of controls.

Note :
- A new calibration curve must be carried out for each new lot of reagents, after each important maintenance of the analyzer, or when measured values for the quality controls are out of the acceptance range determined for the method.
- Each laboratory can establish its own acceptance ranges, according to the instruments and protocols used.
- Include at least one quality control (at different levels) in each test series.

LIMITATIONS OF THE PROCEDURE:

- The assay is not sensitive to presence of heparin in plasma up to a concentration of at least 1 IU/ml.
- Presence of anti-human Factor X antibodies, when present, in plasma may interfere in the assay.
- When compared with a clotting method, slightly higher Factor X concentrations are measured.
- In order to get the optimal performances of the assay, the procedural instructions must be strictly adhered to.
- For in vitro research use.

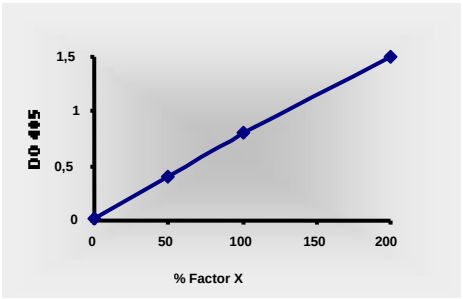
RESULTS:

- For the end point method, using a linear graph paper, plot on abscissa the Factor X concentration (**%**) and on ordinates the corresponding absorbance (**A405**).
- The Factor X concentration in the tested sample is directly obtained on the calibration curve. Results are expressed as % of Factor X.
- Using automated methods, the Factor X concentrations are directly calculated by the analyzer, respectively to the calibration curve.
- The dynamic range is from 5 to 200 %.
- When the assay dilution is **1:10**, the Factor X concentration is directly read on the calibration curve. When different dilutions are used, the results must be multiplied by the dilution factor "**D**", divided by **10**, i.e. **D/10.**

The results obtained should be for research purposes only and not used for patient diagnosis

EXAMPLE OF CALIBRATION CURVE:

The calibration curve below is indicated as an example only. Only the calibration curve generated for the series of measures performed must be used.



VALIDATION OF CALIBRATION CURVE:

The calibration curve is acceptable when linearity is in compliance (r2 ≥0.98) and when the concentrations measured for the Control Plasmas are within the acceptance range.

PERFORMANCES AND CHARACTERISTICS:

- The detection threshold is calculated by measuring the "apparent" A405 obtained for a Factor X deficient sample plus 3 standard deviations (SD). This detection threshold is ≤ 5%.
- The assay is linear up to 200% Factor X.
- Example of Inter-Assay reproducibilities obtained for samples with variable Factor X concentrations (manual method):

Samples	FX concentrations %	Inter-Assay CV%	N
Sample 1	101	5.4	8
Sample 2	58	5.4	8

- The BIOPHEN Factor X assay shows good correlation with a clotting based assay for factor X activity, performed by manual method: Y = 0.87 X n=47 r = 0.98
- In the prestated conditions, the assay is strictly specific for Factor X measurement (use of Russell's viper venom specific action on factor X, absence of phospholipids in the test, presence of specific inhibitors for thrombin (hirudin) and heparin (polybrene)).

BIOCHEMISTRY:

Coagulation Factor X, also called Stuart Factor, is a vitamin K dependant coagulation factor of about 59 kD, synthesized in the liver. Factor X is usually present at about 10µg/ml in plasma, and can largely vary between individuals.

Factor X can be activated by both intrinsic and extrinsic pathways of the coagulation cascade. Prothrombin is converted to thrombin by the action of factor Xa, complexed with factor V in the presence of phospholipids and calcium.

REFERENCES:

1. "Determination of vitamin K sensitive coagulation factors in plasma. Studies on three methods using synthetic chromogenic substrates", Bergström K and Egberg N, Thromb. Res., 12:531-547, 1978.
2. "Activation of decarboxy factor X by a protein from Russell's viper venom. Purification and partial characterization of activated decarboxy factor X", Lindhout MJ, Kop-Klaassen BHM, Hemker MC, Biochim. Biophys. Acta, 533:327-341, 1978.
3. http://www.ncbi.nlm.nih.gov; OMIM; "Coagulation factor X" (+227600).