

BIOPHEN™ Factor X

REF 221705

R1 R2 4 x 2,5 mL, R3 4 x 5 mL

Chromogenic method for plasmatric Factor X assay.
FOR RESEARCH USE ONLY.
DO NOT USE IN DIAGNOSTIC PROCEDURES.



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INTENDED USE:

The BIOPHEN™ Factor X kit is a chromogenic method for *in vitro* quantitative determination of Factor X (FX) on citrated human plasma, using a manual or automated method.

This kit is for research use only and must not be used for patient diagnosis or treatment.

SUMMARY AND EXPLANATION:

Technical^{1,2}:

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

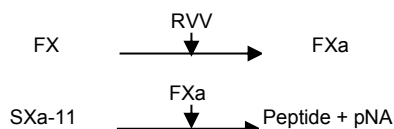
FX can be activated by both intrinsic and extrinsic pathways of the coagulation. Prothrombin is converted to thrombin by the action of FXa, complexed with factor V in the presence of phospholipids and calcium.

PRINCIPLE:

Using the BIOPHEN™ Factor X assay, FX is measured following a specific activation in Activated Factor X (FXa) with RVV (Russell's viper venom), an enzyme extracted from snake venom. 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 FX activity in the tested plasma.

FX concentration present in the specimen to test is therefore directly proportional to the amount of FXa formed, determined by the amount of pNA released, and measured by the optical density at 405nm.



REAGENTS:

R1 Sxa-11 substrate : Chromogenic substrate, specific for FXa (Sxa-11), lyophilized.

R2 RVV (activator): Highly purified enzyme, extracted from the RVV, lyophilized in the presence of calcium and stabilized, can specifically activate Factor X into Factor Xa in presence of calcium.

R3 Tris-NaCl buffer: Ten fold concentrated, liquid form. Contains small amount of sodium azide (0.9 g/L).

R1 R2 4 vials of 2.5 mL

R3 4 vials of 5 mL

The RVV concentration is adjusted for each lot, in order to allow obtaining an optimal reactivity and linearity for the assay.

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of animal origin. 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.
- In contact with lead or copper pipes, sodium azide can generate explosive compounds.
- 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.

REAGENT PREPARATION:

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

R1 R2 Reconstitute the contents of each vial with exactly **2.5 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.

R3 Shake the vial and dilute the amount required **1:10 in distilled water** (the 5 mL contained in the vial allow preparing 50 mL of ready to use buffer after dilution).

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

- 1 month** at 2-8°C.
- 3 days** at room temperature (18-25°C).
- 1 month** frozen at -20°C or less*
- Stability on board of the analyzer: see the specific application.**

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

- 1 week** at 2-8°C.
- 3 days** at room temperature (18-25°C).
- 1 month** frozen at -20°C or less*
- Stability on board of the analyzer: see the specific application.**

*Thaw only once, as rapidly as possible at 37°C and use immediately.

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

- 4 weeks** at 2-8°C.
- 7 days** at 2-8°C, for the diluted solution.
- Stability on board of the analyzer: see the specific application.**

If the substrate become yellow, this indicate a contamination. Discard the vial and use a new one.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- 20% acetic acid or 2% citric acid (end point method).
- Specific calibrators and controls :

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 analyzer for chromogenic assays.
- Stopwatch; Calibrated pipettes; plastic test 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-A5³ guideline for further information concerning specimen collection, handling and storage). For plasma storage, please refer to references⁴.

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**.

Assay method:

1. The calibration curve can be established using a pool of citrated normal plasmas or calibrator plasma with a known FX concentration, C which is by definition 100% of FX.

The assay includes a plasma dilution of 1:10. The calibration range is from 0 to 200% FX. The 200% FX activity is then the 1:5 dilution.

Using a plasma calibrator with a Factor X concentration of C, the 200% FX concentration is obtained (in the assay conditions) by using the following dilution factor: $5 \times C:100$.

The calibration curve can then be prepared as follows from the preparation in **R3** buffer:

Specimen	2C	C	C:2	0
FX (%)	200	100	50	0
Volume Calibrators	500µL	250µL	125µL	0µL
Volume R3 buffer	0µL	250µL	375µL	500µL

2. Dilute the specimens and controls in **R3** buffer, as described in the table below:

Specimens	Référence	Dilution in R3
Controls	223201-RUO / 223301-RUO	1:10
Specimens	NA	1:10

Establish the calibration curve and test it with the quality controls. If 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. Dispense the following to the wells of a microplate, or to a plastic tube incubated at 37°C:

	Microplate	Test tube
Specimen, calibrator or control diluted	50 µL	200 µL
incubate at 37°C for 1-2 minutes, then add the following:		
R1 SXa-11 substrate, Pre-incubated at 37°C	50 µL	200 µL
Mix and incubate at 37°C for 1-2 minutes, then add the following:		
R2 RVV, Pre-incubated at 37°C	50 µL	200 µL
Mix and incubate at 37°C for 2 minutes exactly		
Stop the reaction by adding:		
Citric acid (2%)*	50 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%), R2, R1, diluted specimen.

Measure the optical density at 405 nm. Subtract the measured blank value from the absorbance measured for the corresponding test.

Create a plasma blank if sample is icteric, lipaemic, haemolysed, or if its color differs from the standard plasmas.

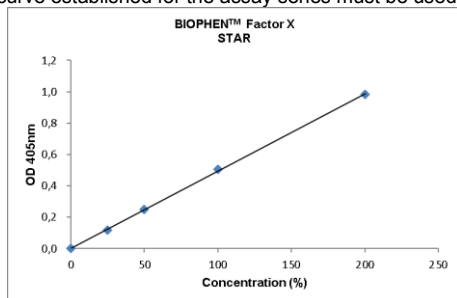
If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

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

CALIBRATION:

The BIOPHEN™ Factor X assay can be calibrated for the assay of FX. The calibrator covering the calibration range is available from HYPHEN BioMed (see the REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED paragraph) and can be used to establish the calibration curve.

The calibration curve shown below 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 established, 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 acceptance range for the method.

Each laboratory must define its acceptance ranges and verify the expected performance in its analytical system.

RESULTS:

- For the manual method, plot the calibration curve lin-lin, with the OD 405 nm along the Y-axis and the FX concentration, expressed as %, along the X-axis.
- When employing the kinetic method, use ΔOD 405 instead of OD 405.
- The concentration of FX (%) in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.
- If other dilutions are used, the level obtained should be multiplied by the additional dilution factor used.

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.
- 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.
- Presence of anti-human FX antibodies in plasma may interfere in the assay.

PERFORMANCES:

- The lower analyzer detection limit depends on the analytical system used ($\leq 5\%$ on manual method).
- The measuring range depends on the analytical system used (about 5 to 200% of FX on manual method).
- In the pretested conditions, the assay is strictly specific for FX (use of RVV specific action on FX, absence of phospholipids in the test, presence of specific inhibitors for thrombin (hirudin) and heparin (polybrene)).
- Performance studies were conducted internally on manual method. The following results were obtained:

Control	Inter assays			
	n	Mean	CV%	SD
Specimen 1	8	101	5.4	5.5
Specimen 2	8	58	5.4	3.1

- Correlation with reference method (BIOPHEN™ Factor X vs FX clotting assay on KC10):
 $n = 47$ $y = 0.87x$ $r = 0.98$
- Interferences:**
No significative interference was observed on Heparin assay up to 1 IU/mL in plasma.

REFERENCES:

- Bergström K and Egberg N. Determination of vitamin K sensitive coagulation factors in plasma. Studies on three methods using synthetic chromogenic substrates. Thromb. Res. 1978.
- Lindhout MJ *et al.* Activation of decarboxy factor X by a protein from Russell's viper venom. Purification and partial characterization of activated decarboxy factor X. Biophys. Acta. 1978.
- CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008
- Mauge L. and Alhenc-Gelas M. Stabilité pré-analytique des paramètres de la coagulation: revue des données disponibles. Ann Biol Clin. 2014.

SYMBOLS:

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

R2 H319 : Causes serious eye irritation.

Changes compared to the previous version.