BIOPHEN™ Factor XIa

REF 220412

R1A R1B R2 R3 2 x 3 mL, R4 2 x 25 mL, CAL 2 x 2 mL

HYPHEN BioMed
155 rue d'Eragny, 95000 Neuville-sur-Oise, France
Tél : +33 (0)1 34 40 65 10
Fax : +33 (0)1 34 48 72 36
www.hyphen-biomed.com
info@hyphen-biomed.com

Chromogenic assay for measuring Factor XIa activity.
FOR RESEARCH USE ONLY.
DO NOT USE IN DIAGNOSTIC PROCEDURES.

English, last revision: 10-2023

INTENDED USE:

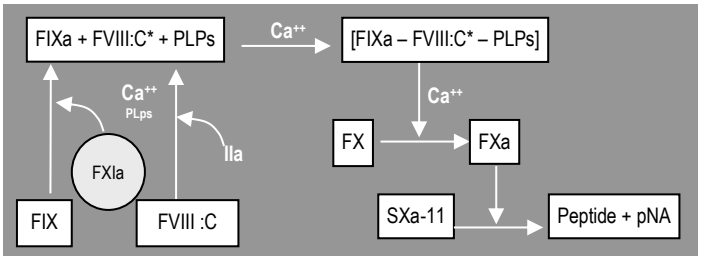
BIOPHEN™ Factor XIa kit is a chromogenic method for *in vitro* quantitative determination of activated Factor XI (FXIa) activity, in purified medium, using an automated or manual method.
This kit is for research use only and must not be used for patient diagnosis or treatment.

SUMMARY AND EXPLANATION:

Technical:[1]
The normal Factor XI (FXI) concentration in human plasma is of about 3 to 7 µg/mL. FXI is present in plasma as a zymogen, and when activated (by FXIIa, thrombin, or autoactivation), it becomes a trypsinlike serine protease which participates in the contact phase of blood coagulation.

PRINCIPLE:

In presence of Phospholipids (PLPs), calcium and thrombin, FXIa present in the tested sample is able to activate FIX into FIXa. FIXa forms an enzymatic complex with thrombin activated Factor VIII:C (FVIII:C), to activate Factor X (FX). The resulting Factor Xa hydrolyzes the chromogenic substrate, leading to the release of paranitroaniline (pNa). The amount of pNA released (measured by absorbance at 405 nm) is directly proportional to the concentration of FXIa in the specimen (FIX, FVIII:C and FX are in constant excess amount).



Note: FVIII:C: Thrombin activated FVIII:C.*

REAGENTS:

R1A **Human Factor X and FVIII:C:** lyophilized. Contains calcium chloride dihydrate, copper sulfate, a fibrin polymerization inhibitor, stabilizers and BSA.
R1B **Human Factor IX (without FIXa):** lyophilized. Contains stabilizers and BSA.
R2 **"Activation" Reagent (Thrombin-Calcium-Phospholipids),** lyophilized. Contains human thrombin, calcium, imidazole, synthetic phospholipids, stabilizers and BSA.
R3 **SXa-11 substrate**: Chromogenic substrate specific for FXa (SXa-11), lyophilized. Contains a FXIa inhibitor.
R4 **Specific Tris-BSA Buffer:** Reaction buffer, ready to use. Contains 1% BSA, PEG.
CAL **FXIa calibrator:** Lyophilized purified human FXIa containing a titrated quantity of FXIa of approximately 45 mIU/mL. Contains stabilizers and BSA.

R1A R1B R2 R3 2 vials of 3 mL
R4 2 vials of 25 mL
CAL 2 vials of 2 mL

The calibrator concentrations may vary slightly from one batch to another. For the assay, see the exact values indicated on the flyer provided with the kit used.

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.
- 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.

R1A **R1B** **R2** **R3** Reconstitute the contents of each vial with exactly **3 mL of distilled water.**
Shake vigorously until complete dissolution while avoiding formation of foam and load it 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.

CAL Reconstitute the contents of each vial with exactly **2 mL of distilled water.**
Shake vigorously until complete dissolution while avoiding formation of foam and load it on the analyzer following application guide instruction.
For manual method, allow to stabilize for 15 minutes at room temperature (18-25°C), homogenize before use.

R4 Reagent is ready to use; homogenize and load it 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.

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.

R1A **R1B** **R2** Reagent stability after reconstitution, free from any contamination or evaporation, and stored closed, is of:

- **24 hours** at 2-8°C.
- **8 hours** at room temperature (18-25°C).
- **2 months** frozen at -20°C or less*
- **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:

- **1 month** at 2-8°C.
- **7 days** at room temperature (18-25°C).
- **2 months** 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.

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

R4 Reagent stability after opening, free from any contamination or evaporation, and stored closed, is of:

- **7 days** at 2-8°C.
- **Stability on board of the analyzer: see the specific application.**

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

- **24 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.**

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- 20% acetic acid or 2% citric acid (end point method).
- Alternatively, reference material for FXIa (internal or international reference preparation).
- Specific controls:

Product Name	Reference
BIOPHEN™ FXIa Control Set	224801

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:

FXIa in purified milieu or in FXI concentrate.

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. Reconstitute the calibrators and controls as indicated in the specific instructions or according to internal practice. Calibrator should be diluted in the **R4** buffer as described in the table below in order to prepare the calibration curve ("C" defines the concentration of FXIa):

Calibrator	C	C:2	C:4	C:8	0
Volume of calibrator at C	1mL	0.5mL	0.25mL	0.125mL	0 mL
Volume Buffer **R4**	0mL	0.5mL	0.75mL	0.875mL	1mL

The calibration curve can also be established from a FXIa titrated reference material (international or internal standard).
Dilute this material with **R4** buffer, to obtain the "C" FXIa concentration of about **45 mIU/mL**, and prepare the calibration range in **R4** buffer as described previously.

2. Tested specimens and concentrates must be assayed undiluted or diluted in **R4** in order to have a FXIa concentration **≤ 45mIU/mL** in the assayed dilution.
As the assay is performed in presence of Calcium (**R2**), special attention is required if the assayed specimen contains citrate or Na_2EDTA (e.g. sufficient dilution of sample in order to not interfere with the calcium needed for the assay. Alternatively, neutralization of citrate and EDTA ions might be done. The choice is made under each laboratory responsibility).

To assay FXIa in FXI concentrates, the tested specimen must be pre-diluted in **R4** buffer, in order to target a FXIa concentration in the range of about 5 to 35 mIU/mL.
The measured concentration should then be multiplied by the "pre-dilution" factor.

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. Into the microplate well, or into the plastic tube, incubated at **37°C**, introduce:

	Microplate	Volume
Specimens, controls or calibrators diluted in **R4**	50 µL	200 µL
R1A Human Factor X and FVIII:C	50 µL	200 µL
R1B Human Factor IX	50 µL	200 µL
Mix and incubate at **37°C for 2 minutes,** then add the following:		
R2 Activator reagent	50 µL	200 µL
Mix and incubate at **37°C for 2 minutes,** then add the following:		
R3 SXa-11 substrate pre-incubated at **37 °C**	50 µL	200 µL
Mix and incubate at **37°C for 5 minutes,** exactly:		
Stop the reaction by adding the following:		
Citric acid (2%)*	50 µL	200 µL
Mix and measure the optical density at **405 nm** 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, R2, R1B, R1A, diluted specimen.

Measure the optical density at 405 nm. Subtract the measured blank value from the absorbance measured for the corresponding test.
Create a blank if specimen color differs from the standards.

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.

Kinetics method:

The assay can be performed by the kinetics method by measuring the change in absorbance between 10 and 100 seconds after adding the substrate (ie ΔA405). In this case, there is no need to subtract the specimen blank, or to stop the reaction.

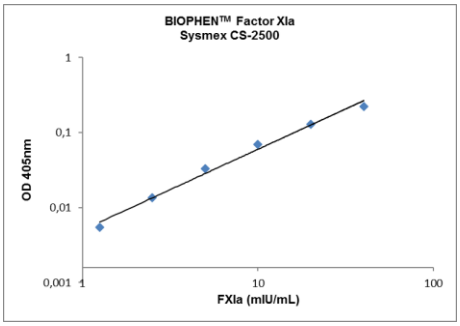
For an automated method, application guides are available on request. See specific application guide and specific precautions for each analyzer.

CALIBRATION:

The BIOPHEN™ Factor XIa assay can be calibrated for the assay of FXIa. The calibrator **CAL** can be used to establish the calibration curve.

- The calibration range is about 1.25 to 40 mIU/mL (on Sysmex CS-2400/2500).

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.

TRACEABILITY:

The FXIa concentration of the FXIa calibrator provided in the kit is exactly defined against the reference International Standard for FXIa, human (NIBSC).

RESULTS:

- For the manual endpoint method, plot the calibration curve log-log, with the OD 405 nm along the Y-axis and the FXIa concentration, expressed as mIU/mL, along the X-axis.
- When employing the kinetic method, use ΔOD 405 instead of OD 405.
- The concentration of FXIa (mIU/mL) 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.

PERFORMANCES:

- The lower analyzer detection limit depends on the analytical system used.
- The measuring range depends on the analytical system used (about 2.5 to 40 mIU/mL of FXIa on Sysmex CS-2400/2500).
- The detection threshold is evaluated on the calibration curve, by measuring the "apparent" FXIa concentration, which corresponds to the mean OD value obtained for a specimen free of FXIa plus 3 Standard Deviations (SD). This detection threshold is **<2.5mIU/mL.**
- Performance studies were conducted internally on Sysmex CS-2400/2500. Performance was assessed using laboratory controls. The following results were obtained:

Control	Intra assay			
	N	Mean	CV%	SD
Level 1	10	36.2	0.7	0.27
Level 2	10	11.2	2.0	0.22

REFERENCES:

1. Bassem MM. *et al.* An Update on Factor XI Structure and Function. Thromb. Res. 2018.

SYMBOLS:

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

R2 H314 : Causes severe skin burns and eye damage.
H318 : Causes serious eye damage.
H360D : May damage the unborn child.

Changes compared to the previous version.