BIOPHEN® Factor XIa

Ref 220412

Chromogenic assay for measuring Factor XIa activity.

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

Last revision: 2016/03/07

INTENDED USE:

BIOPHEN® Factor XIa kit is a chromogenic assay for measuring activated Factor XI (FXIa) activity, using a chromogenic method, manual or automated, through Factor IX activation and Factor Xa generation.

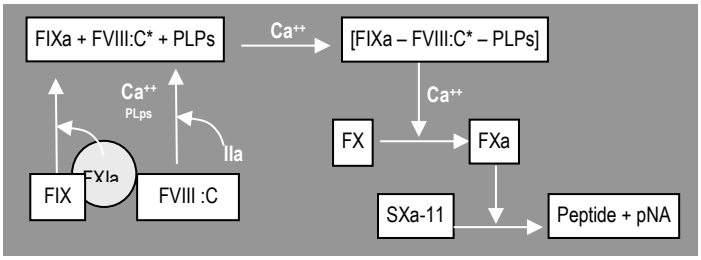
This kit is for research use only and should not be used for patient diagnosis or treatment.

SUMMARY AND EXPLANATION:

The normal Factor XI concentration in human plasma is of about 3 to 7 µg/mL. Coagulation Factor XI (FXI) is a **160KDa** protein synthesized in the liver composed of disulphide linked dimer with identical polypeptide chains. 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. FIX is activated to FIXa by factor XIa, in the presence of calcium, thrombin and phospholipids, and it forms an active complex with thrombin-activated FVIII:C, which is then able to convert FX into FXa.

ASSAY PRINCIPLE:

In presence of Phospholipids (PLPs), calcium and thrombin, activated FXI present in the tested sample is able to activate Factor IX (in excess and without Factor IXa) into FIXa, that forms an enzymatic complex with its cofactor, Factor VIII:C, also supplied in the assay at a constant concentration and in excess. This complex activates Factor X, present in the assay system, into Factor Xa, which generated amount is directly related to the amount of Factor XIa to be measured. Generated Factor Xa is then measured by its specific activity on a Factor Xa chromogenic substrate (SXa-11). Factor Xa cleaves the substrate and releases pNA. Finally, there is a direct relationship between the amount of Factor XIa in the assayed sample and the Factor Xa activity generated, measured by the amount of pNA released, and measured at 405nm.



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

Tested specimen: Purified milieu where Factor XIa needs to be measured or Factor XI concentrates.

REAGENTS:

R1A: Reagent 1A: Human Factor X and FVIII:C

Human Factor X, and FVIII:C, lyophilized in presence of a fibrin polymerization inhibitor and stabilizers.

2 vials (each to be reconstituted with **3 mL** of distilled water).

R1B: Reagent 1B: Human *Factor* IX (without Factor IXa)

Human Factor IX, lyophilized in presence of stabilizers.

2 vials (each to be reconstituted with **3 mL** of distilled water).

R2: Reagent 2: "Activation" Reagent (Thrombin-Calcium-Phospholipids)

Human thrombin, calcium and synthetic phospholipids, lyophilized, in presence of stabilizers.

2 vials (each to be reconstituted with **3 mL** of distilled water).

R3: Reagent 3: SXa-11 (Sequence: Suc-Ile-Glu-(γPip)Gly-Arg-pNA, HCl)

Chromogenic substrate, specific for Factor Xa (SXa-11), lyophilized.

2 vials of SXa-11 (each to be reconstituted with **3 mL** of distilled water).

R4: Reagent 4: Specific Tris-BSA Buffer

Specific Tris-BSA Buffer, ready to use. Contains 1% BSA and sodium azide.

2 vials of **25 mL** each.

Cal: FXIa calibrator: (established against highly purified human FXIa)

Purified human Factor XIa, lyophilized. When restored with **2 mL of distilled water**, a solution containing a concentration "**C**" (expressed in **mIU/mL**) of human FXIa is obtained. This concentration (usually close to **50mIU/mL** according to the lot), is accurately determined for each lot.

2 vials (each to be reconstituted with **2 mL** of distilled water).

The exact concentration of FXIa is indicated on the **flyer** provided in each kit. The calibration curve covers the range from **0** to about **50mIU/mL FXIa**.

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

CAUTIONS AND WARNINGS:

- Any product of biological origin must then be handled with all the required cautions, as being potentially infectious.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.
- 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.
- 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.
- R1A, R1B, R2, R3 and Cal vials are closed under vacuum. Remove carefully the stopper, in order to avoid any loss of powder when opening the vials.
- In order to improve stability, reagents must be closed with their original screw cap following each use (white caps for R1A, R1B, and R2, yellow cap for R3, white cap for buffer R4, and blue cap for Cal).
- Incubating the reconstituted vials at RT allows stabilizing the reagents, and obtaining a homogeneous reactivity.
- Stability studies for 3 weeks at 30°C show that the reagents can be shipped at room temperature for a short period without damage.

PREPARATION AND STABILITY OF REAGENTS:

R1A: Reagent 1A: **Human Factor X, FVIII:C and fibrin polymerization inhibitor:**

Reconstitute each vial with exactly **3 mL** of distilled water, shake thoroughly for complete homogenization.

Before each use, let the reagent stabilize for 30 min at room temperature (18-25°C); while shaking the vial from time to time.

Homogenize before each use.

R1B: Reagent 1B: **Human Factor IX:**

Reconstitute each vial with exactly **3 mL** of distilled water, shake thoroughly for complete homogenization.

Before each use, let the reagent stabilize for 30 min at room temperature (18-25°C); while shaking the vial from time to time.

Homogenize before each use.

R2: Reagent 2: **Thrombin, Phospholipids and Calcium:**

Reconstitute each vial with exactly **3 mL** of distilled water, shake thoroughly for complete homogenization.

Before each use, let the reagent stabilize for 30 min at room temperature (18-25°C); while shaking the vial from time to time.

Homogenize before each use.

Stability of reagent R1A, R1B and R2, provided that any contamination or evaporation is avoided, kept in its original vial or in a closed plastic microcentrifuge tube:

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

Thaw once as rapidly as possible at 37°C, adapt duration to the volume of reagent. The stability of the thawed reagent should be verified in the working conditions of the user laboratory.

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

Reconstitute each vial with exactly **3 mL** of distilled water, shake thoroughly for complete homogenization.

Before each use, let the reagent stabilize for 30 min at room temperature (18-25°C); while shaking the vial from time to time.

Homogenize before each use.

Stability of reagent, provided that any contamination or evaporation is avoided, kept in its original vial or in a closed plastic microcentrifuge tube:

- **1 month** at 2-8°C.
- **7 days** at room temperature (18-25 °C).
- **2 months** frozen at -20°C or below

Thaw once as rapidly as possible at 37°C, adapt duration to the volume of reagent. The stability of the thawed reagent should be verified in the working conditions of the user laboratory.

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

Ready to use buffer. Shake before use.

Stability of the **buffer**, protected from any bacterial contamination:

- In its original vial, until the expiration date printed on the label, at 2-8°C.
- When open, **7 days at 2-8 °C**

Cal: FXIa calibrator:
Reconstitute each vial with exactly **2 mL** of distilled water, shake thoroughly for complete homogenization. A ready to use solution containing a concentration "**C"** (expressed in mIU/mL) of human FXIa is obtained. Incubate at room temperature (18-25°C) for 15 minutes, while shaking the vial from time to time. Homogenize before each use.
Stability of restored calibrator, provided that any contamination or evaporation is avoided, kept in its original vial or in a closed plastic microcentrifuge tube:

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

STORAGE CONDITIONS:

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

TRACEABILITY ON CALIBRATOR MATERIAL:

BIOPHEN® FXIa Calibrator is calibrated against internal reference standard qualified against the NIBSC standard in force.

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water, preferentially sterile.
- 20% acetic acid or 2% citric acid (end point method).
- Quality controls at low and high FXIa concentrations such as BIOPHEN® FXIa Control Set (#224801).
- Alternatively, reference material for factor XIa (internal reference preparation)

Materials:

- Spectrophotometer, photometer or automatic instrument for chromogenic assays.
- Stopwatch; Calibrated pipettes.

TEST PROCEDURE:

The BIOPHEN® Factor XIa kit can be used for kinetics methods, automated on instruments, and can also be used for end point methods. The assay is performed **at 37°C** and the color developed is measured **at 405 nm**.

Automated methods:
Applications to the various analyzers are available upon request. Refer to each specific applications and specific cautions for each instrument.

Assay method:
1. Reconstitute the calibrator and controls using the specific package insert. Calibrator should be diluted using R4 buffer as described in the table below:

FXIa concentration (mIU/mL)	C	C:2	C:4	C:8	0
Volume of FXIa calibrator at C	1mL	0.5mL	0.25mL	0.125mL	0 mL
Volume Buffer R4	0mL	0.5mL	0.75mL	0.875mL	1mL

The calibrator dilutions are stable for at least 4 hours at room temperature (18 – 25°C)
Alternatively, the calibration curve can also be performed using a reference Factor XIa material.
Dilute the required FXIa preparation with **R4** dilution buffer, for obtaining the **"C" mUI/mL** FXIa concentration of about **50mIU/mL,** and prepare the calibration range as for a calibrator titrating **"C" IU/mL** FXIa.
2. Tested samples and concentrates must be assayed undiluted or diluted in **R4** in order to have a FXIa concentration **≤ 50mIU/mL** in the assayed dilution.
As the assay is performed in presence of Calcium (R2), if the assayed specimen contains citrate or Na_2EDTA, it must be diluted enough in order to not interfere with the calcium needed for the assay**.** Alternatively, they can be neutralised with the use of the appropriate concentration of divalent anions.
For FXI concentrates, the tested specimen must be pre-diluted in **R4** buffer, in order to have an expected FXIa concentration within the dynamic range (<50mIU/mL).
The measured FXIa concentration must then be multiplied by the dilution factor (i.e., **x 10** for a sample tested at the **1:10** dilution in R4 buffer).
Please note that the exact concentration of calibrator and controls is indicated for each lot on the flyer provided with the kit.
3. Into the microplate well or into the plastic test tube, incubated at **37°C**, introduce:

	Microwell	Test tube
Specimen diluted, calibrator or controls	50µL	200µL
R1A: Factor X-VIII:C	50µL	200µL
R1B: Factor IX	50µL	200µL
Mix and incubate at 37°C, for **2 minutes**, then introduce:		
R2: activate mixture	50µL	200µL
Mix and incubate at 37°C, for **2 minutes**, then introduce:		
R3: SXa-11 Substrate preincubated at **37°C**	50µL	200µL
Mix and incubate at 37°C, for **5 minutes**, exactly		
Stop the reaction by introducing:		
Citric acid (2%)*	50µL	200µL
Mix and measure the absorbance at **405nm** against the corresponding blank.		

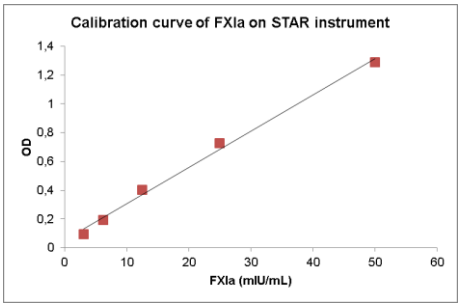
*Or acetic acid (20%). The yellow color is stable for 2 hours.
The sample blank is obtained by mixing the reagents in the opposite order from that of the test i.e., Citric Acid (2%), R3 substrate, diluted sample, R2, R1B, R1A.
Measure the absorbance **at 405 nm**. The sample blank value must be deduced from the absorbance measured for the corresponding assay.

If an other reactive mixture volume than this indicated here above is required for the method used, the volumes ratio must be strictly adhered to, in order to maintain the assay performances. It is responsibility of the user to validate any modifications and their impact on all assay results.

Kinetics mode:
The assay can be read using an end point method as depicted here above, or a kinetics mode. In this case the change in absorbance is recorded from 10 seconds to 100 seconds following the addition of substrate. There is then no need to subtract the sample blank, or to stop the reaction. The results are obtained from the measurement of the change in absorbance (ΔA405) for calibrators and tested specimen during the reading window time.

CALIBRATION:

The BIOPHEN® Factor XIa assay can be calibrated for measurement of XIa. FXIa calibrator which cover the assay's dynamic range can be used to generate calibration curves.
The calibration curve below, obtained with the calibrator is indicated as an example only. The calibration curve generated for the series of measures performed must be used.



QUALITY CONTROL:

Using quality control 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 generated 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 should establish and verify its own target values, acceptance ranges and expected performances, according to the instruments and protocols used.

RESULTS:

- For the end-point method, use a **bilogarithmic** graph paper and plot on abscissae the Factor XIa concentration **(mUI/mL**) and on ordinates the corresponding absorbance (**A405**).
- Alternatively, statistics software can be used for establishing the dose response calibration curve.
- Draw the calibration curve obtained. Calculate the **"r^2"** value. Calibration is acceptable if: **$r^2 \geq 0.98$**, and if measured values for controls are in compliance. Alternatively the Akima expression mode can be used for the calibration curve (no r^2 can then be calculated).
- The Factor XIa concentration in the diluted tested sample is directly obtained on the calibration curve. Results are expressed as **mUI/mL Factor XIa**. When diluted specimen is assayed, multiply the measured Factor XIa concentration by the dilution factor in order to get the FXIa concentration.
- When the kinetics mode is used, proceed the same way by plotting the **ΔA405** values obtained, instead of A405.
- Using automated methods, the Factor XIa concentrations are directly calculated by the analyser, respectively to the calibration curve, and the sample dilution used.

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

LIMITATION:

- In order to get the optimal assay performances, the working instructions, as well as the working temperature at 37°C, must be carefully observed.
- 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 R1A, R1B, R2 and R3 must be adhered to.

PERFORMANCE:

- The assay allows measuring **up to about 50mIU/mL** FXIa in the assayed dilution.
- The detection threshold for the assay is evaluated on the calibration curve by measuring the "apparent" Factor XIa concentration, which corresponds to the mean A405 value obtained for a sample free of Factor XIa plus 3 Standard Deviations (**3 SD**s). This detection threshold is **<2.5mIU/mL.**

REFERENCES:

1. Woodhams B, Girardot O, Blanco M-J, Colesse G, Gourmelin Y.Stability of coagulation proteins in frozen plasma. Blood coagulation and Fibrinolysis. 2001. Vol 12, No 4. 229-236.

SYMBOLS:

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