# BIOPHEN™ Factor IXa

**REF 221812**

**R1 R2 R3 2 x 2.5 mL, R4 2 x 25 mL, CAL 2 x 1 mL**

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

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English, last revision: 08-2019

## INTENDED USE:

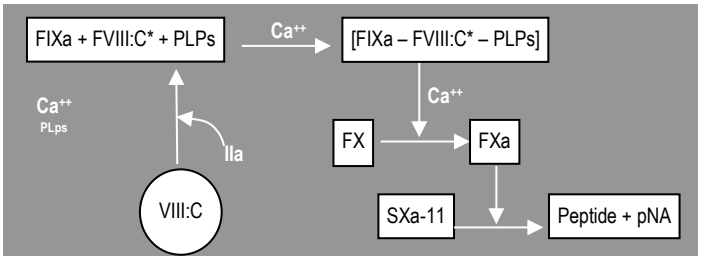
BIOPHEN™ Factor IXa kit is a chromogenic method for *in vitro* quantitative determination of activated Factor IX (FIXa) activity, in purified medium, using an automated or manual method.
**This kit is for research use only and must not be used for patient diagnosis or treatment.**

## SUMMARY AND EXPLANATION:

***Technical:***
Factor IX (FIX) is a vitamin K-dependent glycoprotein involved in the intermediate phases of coagulation. Its normal concentration in human plasma is of 4 to 5 µg/mL[1]. When activated by FXIa in the presence of calcium, FIXa forms an active complex with Factor VIII: C (FVIII:C), in the presence of calcium and phospholipids, thus activating Factor X to Factor Xa (FXa)[2].

## PRINCIPLE:

In presence of Phospholipids (PLPs) and Calcium, activated FIXa, present in the tested sample, forms an enzymatic complex with thrombin activated FVIII:C, to activate Factor X.
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 Factor IXa in the specimen (FVIII:C and Factor X are in constant excess amount).




*Nota : FVIII:C*: Thrombin activated FVIII:C*

## REAGENTS:

**R1 FX(h)-FVIII:C:** Human Factor X and lyophilized FVIII:C. Contains calcium chloride dihydrate, copper sulfate, a fibrin polymerization inhibitor, stabilizing agents and BSA.
**R2 Activator reagent:** lyophilized. Contains human thrombin, calcium chloride dihydrate, imidazole, synthetic phospholipids, stabilizing agents and BSA.
**R3 Substrate:** Lyophilized chromogenic substrate specific to FXa (SXa-11). Contains a FXIa inhibitor.
**R4 Buffer:** Tris-BSA reaction buffer. Contains 1% BSA, PEG, FVIII:C stabilizing agents and small amounts of sodium azide (0.9 g/L) as a preservative.
**CAL FIXa calibrator**: Lyophilized purified human FIXa containing a titrated quantity of FIXa of approximately 20 mUI/mL.

**R1 R2 R3 2 vials of 2.5 mL.**
**R4 2 vials of 25 mL.**
**CAL 2 vials of 1 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.
- 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 R3** 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.*

**CAL** Reconstitute the contents of each vial with exactly **1 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 15 minutes at room temperature (18-25°C), homogenize before use.*

**R4** Reagent is ready to use; homogenize 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.*

## 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 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.**

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

**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.

**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.**

If the substrate becomes 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 controls:

| Product Name | Reference |
|---|---|
| **BIOPHEN™ FIXa Control Set** | 224601 / 224601-C2 |

Also refer to the specific application guide of the analyzer used.

***Materials:***
- Spectrophotometer or automatic analyzer for chromogenic assays.
- Stopwatch; calibrated pipettes; silicon glass or plastic test tubes or microplate.

## SPECIMEN:

Factor IXa in purified milieu or in FIX concentrate.

## PROCEDURE:

The kit can be used for kinetic, automated or manual (endpoint) methods. Perform the test at **37 °C** and read color intensity at **405 nm**.

**Assay method:**

1. Reconstitute the controls as indicated in the specific instructions. For the calibration curve, dilute the calibrator in 1:2 in R4 buffer to get the "C" concentration (approximately 20 mIU/mL), then prepare the calibration curve as described below ("C" defines the concentration of FIXa):

| Calibrator | C | C:2 | C:4 | C:10 | C:20 | 0 |
|---|---|---|---|---|---|---|
| Volume of calibrator at C | 1mL | 0.5mL | 0.25mL | 0.125mL | 0.050mL | 0mL |
| Volume of R4 buffer | 0mL | 0.5mL | 0.75mL | 0.875mL | 0.950mL | 1mL |

Prepare the calibration curve immediately before use to avoid any FIXa degradation.

The calibration curve can also be established from a FIXa titrated reference material (international standard or internal standard).
Pre-dilute this material at least 1:2 in **R4** buffer for obtaining the "C" mIU/mL FIXa concentration and prepare the calibration range in R4 buffer as previously explained.

2. Dilute the specimens in R4 buffer, as described in the table below:

| Specimens | Reference | Dilution |
|---|---|---|
| Control | 224601 / 224601-C2 | **1 :2** |
| Specimens | N.A. | **1 :2** |

To measure the FIXa in Factor IX concentrates, the tested specimen must be pre-diluted at least 1:2 in R4 buffer. If required, it is recommended to prepare a pre-dilution, in order to bring the expected FIXa concentration in the range 3 to 15 mIU/mL with R4 buffer, and then dilute it 1:2 with R4 buffer for the assay.
The measured concentration must 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), the diluted samples should be tested quickly. The exact calibrator and controls 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 | Volume |
|---|---|---|
| Specimens, controls or calibrators diluted in **R4** | 50 µL | 200 µL |
| **R1** **FX(h)-VIII:C** pre-incubated at **37°C** | 50 µL | 200 µL |
| Mix and incubate at **37°C for 2 minutes,** then add the following: | | |
| **R2** **Activator reagent** pre-incubated at **37°C** | 50 µL | 200 µL |
| Mix and incubate at **37°C for 3 minutes,** then add the following: | | |
| **R3** **Substrate SXa-11** pre-incubated at **37 °C** | 50 µL | 200 µL |
| Mix and incubate at **37°C for 3 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, R1, dilute 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.
When employing the kinetic method, use ΔOD 405 instead of OD 405.

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.

**Kinetic method:**

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

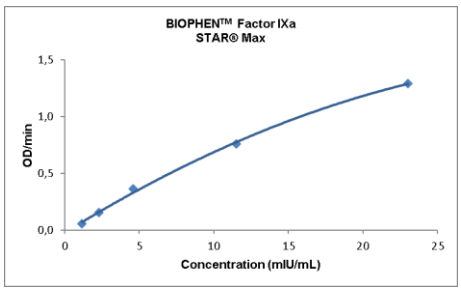
**For an automated method, application guides are available on request. See specific application guide and specific precautions for each analyzer.**

## CALIBRATION:

The BIOPHEN™ Factor IXa assay can be calibrated for the assay of FIXa. The calibrator can be used to establish the calibration curve.

- The calibration range is about 1 to 20 mIU/mL (on STAR).

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 FIXa concentration of the FIXa calibrator provided in the kit is exactly defined against the reference International Standard for FIXa, 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 concentration of FIXa, expressed as (mIU/mL), along the X-axis.
- When employing the kinetic method, use ΔOD 405 instead of OD 405.
- The concentration of FIXa (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 1 to 20 mIU/mL of FIXa on STA-R®-series.
- The detection threshold for the assay is evaluated on the calibration curve by measuring the "apparent" FIXa concentration, which corresponds to the mean OD value obtained for a sample free of FIXa plus 3 Standard Deviations (SD). This detection threshold is of about **0.1 mIU/mL** (i.e., about 0.1 ng/mL).
- Performance studies were conducted internally on STA-R® Max. The following results were obtained:

| Controls | Intra assay | | | |
|---|---|---|---|---|
| | n | Mean | CV% | SD |
| Control 1 | 10 | 8.8 | 2.9 | 0.3 |
| Control 2 | 10 | 19.1 | 0.9 | 0.2 |

## REFERENCES:


1. Lowe G.D.O. *et al.* Epidemiology of coagulation factors, inhibitors and activation markers : The third glasgow MONICA survey I. Illustrative reference ranges by age, sex and hormone use. British Journal of Haematology, 1997.
2. Taran LD. Factor IX of the blood coagulation system: a review. Biochemistry (Mosc.) 1997.
3. Kitchen S. *et al.* A computer-based model to assess costs associated with the use of factor VIII and factor IX one-stage and chromogenic activity assays. J Thromb Haemost 2016.


## SYMBOLS:

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

**R1**
H315 : Causes skin irritation.
H319 : Causes serious eye irritation.
H335 : May cause respiratory irritation.
H412 : Harmful to aquatic life with long lasting effects.

**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.*