BIOPHEN™ FIX

REF 221801 R1 R2 R3 2 x 1 mL; R4 2 x 15 mL
REF 221802 R1 R2 R3 2 x 2,5 mL; R4 2 x 25 mL
REF 221806 R1 R2 R3 2 x 6 mL; R4 4 x 25 mL

CE
IVD

Chromogenic method for the assay of Factor IX in plasma or therapeutic concentrates

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INTENDED USE:

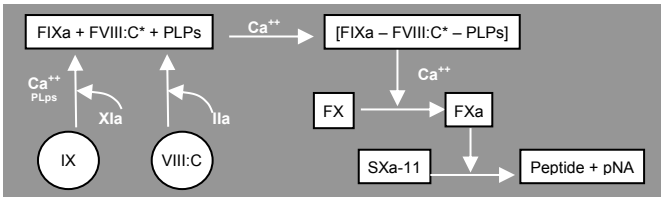
The BIOPHEN™ FIX kit is a chromogenic method for the *in vitro* quantitative determination of Factor IX activity on citrated human plasma or therapeutic concentrates, based on an automated or manual amidolytic method.

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 Factor XIa in the presence of calcium, Factor IX(a) forms an active complex with Factor VIII: C, in the presence of calcium and phospholipids, thus activating Factor X to Factor Xa[2].
The BIOPHEN™ FIX kit is used to assay Factor IX activity on plasma or therapeutic concentrates[3,4].
Clinical:
A Factor IX (or antihemophilic factor B) deficiency leads to the hemophilia B disease, a congenital coagulation disorder[5,6,7,8,9]. Factor IX levels are reduced in patients receiving anti-vitamin K treatment, or in diseases such as liver disorders, cirrhosis or DIC. High Factor IX concentrations may be suggestive of an increased risk of venous thrombosis[10].

PRINCIPLE:

The BIOPHEN™ FIX method involves the chromogenic assay of Factor IX (FIX) activity. In the presence of phospholipids (PLPs) and calcium, Factor XIa (FXIa) activates the FIX present in the test specimen, converting it to activated Factor IX. Factor VIII:C, activated (FVIIIa) by thrombin, forms an enzyme complex with Factor IXa 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 IX in the specimen (Factor XIa, Factor VIII:C and Factor X are in constant excess amount).



NB: FVIII:C: FVIII:C activated by thrombin.*

REAGENTS:

R1 Reagent 1: FX(h)-FVIII:C: Human Factor X and lyophilized FVIII:C. Contains calcium chloride dihydrate, copper sulfate, a fibrin polymerization inhibitor and stabilizing agents.
R2 Reagent 2: Activator reagent: lyophilized. Contains a constant and optimized amount of human Factor XIa, human thrombin, calcium chloride dihydrate, imidazole, synthetic phospholipids and stabilizing agents.
R3 Reagent 3: Substrate: Lyophilized chromogenic substrate specific to Factor Xa (SXa-11). Contains a Factor XIa inhibitor.
R4 Reagent 4: Buffer: Tris-BSA reaction buffer. Contains 1% BSA, PEG, Factor VIII:C stabilizing agents and small amounts of sodium azide (0.9 g/L) as a preservative.

REF 221801 ➔ R1 R2 R3 2 vials of 1 mL.
R4 2 vials of 15 mL.
REF 221802 ➔ R1 R2 R3 2 vials of 2.5 mL.
R4 2 vials of 25 mL.
REF 221806 ➔ R1 R2 R3 2 vials of 6 mL.
R4 4 vials of 25 mL.

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* diagnostic 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 :
REF 221801 ➔1 mL of distilled water.
REF 221802 ➔2.5 mL of distilled water.
REF 221806 ➔6 mL of distilled water.
Shake vigorously until **complete dissolution** (ensure that there is no deposit at the bottom of the R3 vial) 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.

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.

REF 221802 / 221806 :
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.**

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. Proceed to a new calibration with frozen reagent.

REF 221801 :
R1 R2 R3 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).
- **Stability on board of the analyzer: see the specific application.**

REF 221801 / 221802 / 221806 :
R4 In its original packaging and stored at 2-8°C, the reagent is stable until the expiry date printed on the kit, excluding contamination or evaporation.

A yellow color indicates a contaminated substrate. 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 (endpoint method).
- Reference materials for Factor IX assay in therapeutic concentrates (international or internal).
- Specific calibrators and controls with known titration, such as:

Product name	Reference
BIOPHEN™ Plasma Calibrator	222101
BIOPHEN™ Normal Control Plasma	223201
BIOPHEN™ Abnormal Control Plasma	223301

- For low-range calibration, dilute the calibrator in Factor IX deficient plasma (DP050A/K).

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

Materials:
- Spectrophotometer or chromogenic assay analyzer.
- Stopwatch, Calibrated pipettes.

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-A5[11] guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references[12].

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**.

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

Assay method:
1. Reconstitute the calibrators and controls as indicated in the specific instructions. For the calibration curve, dilute the calibrators in R4 buffer as described below ("C" defines the concentration of Factor IX):

High range (5 to 200%):
When the calibration curve is established using a commercial calibrator plasma (e.g.: BIOPHEN™ Plasma Calibrator), the **1/100** dilution corresponds to the indicated concentration (**C**) of Factor IX and the **1/50** dilution to twice this concentration. For a calibrator with a titer of **C**, the 200% level (under assay conditions) is obtained by diluting this calibrator by the following factor: **50x(C)/100**.
The calibration curve can also be established using a pool of citrated normal plasmas (at least 30 normal individuals, men and women, aged between 18 and 55 years, with no known

treatments or diseases), which, by definition, has a Factor IX titer of **100%**. The assay includes a **1/100** plasma dilution, which by definition, represents the 100% Factor IX level. The calibration curve ranges from **5 to 200%** Factor IX. The **1/50** dilution in R4 buffer represents **200%** Factor IX.

Prepare **2 mL** of the **1/50** normal plasma pool dilution, or a **(50xC/100)** dilution of the Factor IX titrated calibrator plasma (i.e. **C1**). This solution has a Factor IX titer of 200%. Prepare the following calibration curve by serial dilution in R4 buffer, as described in the following table:

Calibrator	C1	C2	C3	C4	C5	0
FIX(%)	200	100	50	25	5	0
Volume of calibrator	1000 µL of C1	500 µL of C1	500 µL of C2	500 µL of C3	100 µL of C4	0 µL
Volume of R4 buffer	0 µL	500 µL	500 µL	500 µL	400 µL	500 µL

The calibration curve can also be established from a Factor IX titrated reference material (international standard or internal standard).
Pre-dilute this material in **R4** buffer to obtain a **1IU/mL** solution, then dilute **1/50** in **R4** to obtain a solution with a **200%** (2 IU/mL) Factor IX titer. Use this solution to establish a calibration curve in R4 buffer as previously explained.

Low range (0 to 20%):
Calibration can be performed using a pool of citrated normal plasmas, or a commercial calibrator plasma with a known concentration of Factor IX, i.e. **C**. Dilute this plasma in Factor IX-deficient plasma (DP050A/K) to achieve a **20%** concentration (the dilution factor in deficient plasma is of **5** for the normal pool and of **5xC/100** for a calibrator with a concentration **C**).
The assay method includes a **1/20** plasma dilution. The calibration curve ranges from **1 to 20%** Factor IX. The **1/20** dilution in **R4** buffer represents **20%** Factor IX.

Using this solution, establish the following calibration curve in R4 buffer:

FIX(%)	20	10	5	2.5	1	0
Volume of 20% FIX Calibrator	500 µL	250 µL	125 µL	65 µL	25 µL	0 µL
Volume of R4 buffer	0 µL	250 µL	375 µL	455 µL	475 µL	500 µL

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

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

Specimens	Reference	Range	Dilution
Control	223201/223301	High	1/100
		Low (after 1/10 pre-dilution in FIX-deficient)	1/20
Specimens	N.A.	High	1/100
		Low	1/20

For Factor IX therapeutic concentrates, pre-dilute the test specimen (**high range**) in R4, aiming for a Factor IX concentration of approximately 1 IU/mL. We recommend performing a pre-dilution, in order to adjust the theoretical Factor IX concentration to **between 0.2 and 2 IU/mL**, then dilute **1/100** in R4 to perform the test. The expected Factor IX concentration is thus of between 20 and 200%.
(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), 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	Test tube
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 2 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 plasma blank if this latter is icteric, lipaemic, haemolysed, or if its color differs from the standard plasmas.
When employing the kinetic method, use ΔOD 405 instead of OD 405.

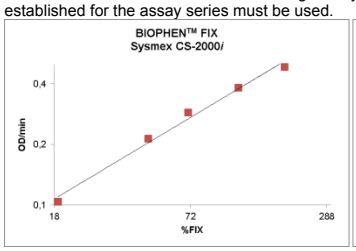
Kinetic method:
The assay can be performed by the kinetic method by measuring the change in absorbance between 10 and 100 seconds after adding the substrate (i.e. ΔA405). In this case, there is no need to subtract the specimen blank, or to stop the reaction.

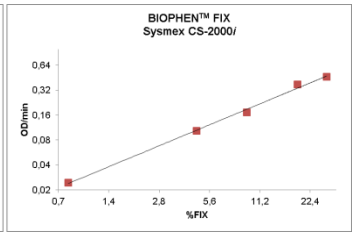
If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

CALIBRATION:

The BIOPHEN™ FIX test can be calibrated for the assay of Factor IX in plasma or therapeutic concentrates. The plasma calibrator covering the dynamic test 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 curves shown below are 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 defined, 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 acceptable range for the method.
Each laboratory must define its acceptable ranges and verify the expected performance in its analytical system.

RESULTS:

- For the manual endpoint method, plot the calibration curve log-log, with the OD 405 nm along the Y-axis and the Factor IX concentration, expressed as a percentage, along the X-axis.
- The concentration of Factor IX (%) in the test specimen is directly inferred from the calibration curve, if the standard dilution is used.
- The results should be interpreted according to the patient's clinical and biological condition.
- If other dilutions are used, the level obtained is the measured level, multiplied by the additional dilution factor used.

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.

EXPECTED VALUES:

The normal Factor IX value for adult plasma is generally between 73 and 167% (Sysmex CS-5100). Each laboratory, however, must establish its own normal interval.

PERFORMANCE:

- The lower analyzer detection limit is less than **2% in high range** and less than **0.5% in low range.**
- The measurement domain on the Sysmex CS-2000*i* is of between **1 and 250%** for the high range and between **0.8 and 30%** for the low range (and generally of between **5 and 200%** for the high range and between **1 and 20%** for the low range).
- Performance studies were conducted internally on 1 batch of reagent using a Sysmex CS-2000*i*. Performance was assessed using laboratory controls over a 20-day period, 2 series per day and duplicates within each series for a control level. The following results were obtained:

Control	High range								Low range			
	Intra-series				Inter-series				Inter-series			
	N	Mean	SD	CV%	n	Mean	SD	CV%	n	Mean	SD	CV%
Normal	30	85.7	1.0	1.2	80	83.1	4.1	4.9	80	8.3	0.5	6.2
Abnormal	30	36.5	0.9	2.4	80	35.7	2.0	5.6	80	3.6	0.2	6.4

- Correlation with reference method (STA® C.K. PREST® on STA-R vs BIOPHEN™ FIX on Sysmex CS-5100) :
  n = 102 y = 0,97x - 0,10 r = 0,983
- Interferences:
  No interference up to (Sysmex CS-2000i, high range):

Intralipids (mg/dL)	Hemoglobin (mg/dL)	Dabigatran (ng/mL)	Bilirubin (F/C) (mg/dL)	Apixaban (ng/mL)	Heparin (UFH/LMWH) (UI/mL)
1000	1000	500	60	50	2

Refer to the specific application guide.

REFERENCES:

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SYMBOLS:

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

R1 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.

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