**BIOPHEN™ FIX**

**REAGENT**

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

**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. When activated by Factor Xa in the presence of calcium, Factor Xa (IXa) forms an active complex with Factor VIII:C, in the presence of calcium and phospholipids, thus activating Factor X to Factor Xa.

The BIOPHEN™ FIX kit is used to assay Factor IX activity on plasma or therapeutic concentrates.

**Clinical:**

A Factor IX (or arthrometabolic factor B) deficiency leads to the hemophilia B disease, a congenital coagulation disorder. 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.

**PRINCIPLE:**

The BIOPHEN™ FIX method involves the chromogenic assay of Factor IX (FIX) activity. In the presence of phospholipids (PLPs) and calcium, Factor X (IXa) activates the FIX present in the test specimen, converting it to activated Factor IX (Factor IXa). Activated FIX (FIXi) is a thrombin-like enzyme complex with Factor IXa to activate Factor X. The resulting Factor Xa hydrolizes the chromogenic substrate, leading to the release of para-nitroanilin (pNPA). The amount of pNPA released (measured by absorbance at 405 nm) is directly proportional to the concentration of Factor IX in the specimen (Factor Xa, Factor VIII:C and Factor X are in constant excess amounts).

**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.

**MANUFACTURER:**

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**PRODUCT NAME:**

BIOPHEN™ FIX

**PRODUCT CODE:**

221801, 221802, 221806

**REAGENT STABILITY:**

• Reactant 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.

**STORAGE ON BOARD OF THE ANALYZER:**

Stability on board of the analyzer: see the specific application.

**STORAGE:**

Aging studies show that the reagents can be stored for 2 months at 2-8°C. 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:**

Stability on board of the analyzer: see the specific application.

**TIME FOR REACTANT STABILITY:**

• 1 day 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:**

Stability on board of the analyzer: see the specific application.

**STORAGE:**

A yellow color indicates a contaminated substrate. Discard the vial and use a new one.

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**REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:**

**Reagents:**

- Distilled water.
- 10 µg/mL acetic acid or 2% citric acid (endpoint method).
- Reference materials for Factor IX assay in therapeutic concentrates (internal or external).
- Specific calibrators and controls with known titration, such as:
  - Free range of calibration, dilute the calibrator in Factor IX deficient plasma (DP050A.K).
  - 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. The blood should be stored in accordance with applicable local regulations. For plasma storage, please refer to references.

**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 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 300% level (under assay conditions) is obtained by diluting this calibrator by the following factor: 50(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

**REF 221801**

Reagent is ready to use: homogenize and load it on the analyzer following application guide instructions.

**REF 221802**

Reagent is ready to use: homogenize and load it on the analyzer following application guide instructions.

**REF 221806**

Reagent is ready to use: homogenize and load it on the analyzer following application guide instructions.

**EAGENT PREPARATION**

• 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 in the analyzer following application guide instructions.
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 (50xCl100) 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 diluting Factor IX in R4 buffer, as described in the following table:

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIX (%)</td>
<td>200</td>
<td>100</td>
<td>50</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Volume of calibrator</td>
<td>500 µL</td>
<td>500 µL</td>
<td>500 µL</td>
<td>500 µL</td>
<td>500 µL</td>
<td>500 µL</td>
</tr>
<tr>
<td>Volume of R4 buffer</td>
<td>0 µL</td>
<td>0 µL</td>
<td>0 µL</td>
<td>0 µL</td>
<td>0 µL</td>
<td>0 µL</td>
</tr>
</tbody>
</table>

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 1/100 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 diluted 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 (D/P050/A/K) to achieve a 20% concentration (the dilution factor in deficient plasma is 5 for the normal pool and of 5x/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:

<table>
<thead>
<tr>
<th>FIX (%)</th>
<th>20</th>
<th>10</th>
<th>5</th>
<th>2.5</th>
<th>1</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of 20% FIX</td>
<td>500 µL</td>
<td>250 µL</td>
<td>125 µL</td>
<td>65 µL</td>
<td>25 µL</td>
<td>0 µL</td>
</tr>
<tr>
<td>Volume of R4 buffer</td>
<td>0 µL</td>
<td>0 µL</td>
<td>0 µL</td>
<td>50 µL</td>
<td>100 µL</td>
<td>200 µL</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Reference</th>
<th>Controls</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>1/100</td>
<td>1/50</td>
<td>1/25</td>
</tr>
<tr>
<td>Low</td>
<td>1/100</td>
<td>1/50</td>
<td>1/25</td>
</tr>
</tbody>
</table>

For Factor IX therapeutic concentrates, pre-dilute the test specimen (high range) in R4, aiming for a Factor IX concentration of approximately 1 U/mL. We recommend performing a pre-dilution, in order to adjust the theoretical Factor IX concentration to between 0.2 and 2 U/mL, then dilute 1/100 in R4 to perform the test. The expected Factor IX concentration is thus between 20 and 200% (0.2 and 2 U/mL).

(1) 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 control concentrations for each batch are indicated on the flyer provided with the kit. When employing the kinetic method, use AOD 405 instead of OD 405.

3. Dispense the following to the wells of a microplate, or to a plastic tube incubated at 37°C:

- **Specimens, controls or calibrators diluted in R4:** Mix and incubate at 37°C for 2 minutes, then add the following:
  - **R4** (200 µL/FX-I/10/20) Pre-incubated at 37°C
  - **R5** (200 µL)

- **R1 Substrate 50x/FX-I/20 pre-incubated at 37°C**: Mix and incubate at 37°C for 2 minutes, then add the following:
  - **Citrate buffer**: Mix and measure the optical density at 405 nm against the corresponding blank.

- **R2 Activator reagent pre-incubated at 37°C**
  - **R3** (100 µL)

- **R3** (100 µL)

Create a plasma blank if this latter is icteric, lipemic, haemolysed, or if its color differs from the standard plasma.

When employing the kinetic method, use AOD 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. A405). 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 between 10 and 100 seconds after adding the substrate (i.e. A405).

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 quality of quality controls serves to validate method compliance, along with between-test assay homogeneity for a given batch of reagents. Indeed, the quality control laboratory may be considered as a quality assurance 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.
- 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.
- 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.

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

**LIMITATIONS:**

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPOST 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-2000i 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 reagents using a reference assay on a Sysmex CS-2000i. 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:

  - **Inter-series:** Ref: 95.9 ± 0.10; r = 0.983
  - **Intra-series:** No interference up to (Sysmex CS-2000i, high range):

- Refer to the specific application guide.

**REFERENCES:**


**SYMBOLS:**

Symbols used and signed listed in the ISO 15184-3 standard, see Symbol definitions document.

**H314:** Causes severe skin burns and eye damage.
**H360D:** May damage the unborn child.

Changes compared to the previous version.