INTENDED USE:
The BIOPHEN™ FVIII:C kit is a chromogenic method for in vitro quantitative determination of Factor VIII activity (FVIII:C) on citrated human plasma or therapeutic concentrates, using a manual or automated amylographic method.

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

SUMMARY AND EXPLANATION:
Technical:
Factor VIII is an approximately 280 kDa protein. It is present in plasma at very low concentrations (100-200 ng/mL). In blood, FVIII is stabilized by its binding to von Willebrand Factor (vWF), which dramatically prolongs its half-life in blood circulation. In the absence of vWF, FVIII activity is rapidly cleared from blood.

PRINCIPLE:
The BIOPHEN™ Factor VIII:C method involves the chromogenic assay of FVIII:C cofactor. In the presence of phospholipids (PLPs) and calcium, FVIII:C, activated by thrombin, forms an enzyme complex with Factor IXa, which activates 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 FVIII:C in the specimen (Factor IXa, Thrombin and Factor X are in constant excess amount).

REAGENT PREPARATION:
• Human Factor IXa, at a constant and optimized concentration, human thrombin, calcium, stabilizing agents.

REAGENTS:
REAGENTS AND MATERIALS PROVIDED:
For low range calibration, dilute the calibrator in FVIII:C deficient plasma (DPP40A/K-RUO). Also refer to the specific application guide of the analyzer used.

Materials:
• Spectrophotometer or automatic instrument for chromogenic assays.
• Stopwatch; Calibrated pipettes, plastic test tubes or microplate.

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

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 calibrator and controls as indicated in the specific instructions. Calibrators should be diluted in the RUO buffer as described in the table below in order to prepare the calibration curve (C" defines the concentration of FVIII:C).

High range (0 to 200%):
When the calibration curve is established using a commercial calibrator plasma (e.g.: BIOPHEN™ Plasma Calibrator), the 1:20 dilution corresponds to the indicated concentration (C) of FVIII:C and the 1:20 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: 20(C) x 100. The calibration curve can also be established using a pool of citrated normal plasma (at least 35 normal individuals, men and women, aged between 18 and 55 years, with no known treatments or diseases), which, by definition, has a FVIII:C titer of 100%. The assay includes a 1:40 plasma dilution, which by definition, represents the 100% FVIII:C level. The calibration curve ranges from 0 to 200% FVIII:C. The 1:20 dilution in RUO buffer represents 200% FVIII:C.

Prepare 1 mL of the 1:20 normal plasma pool dilution, or a (200%C)100 dilution of the FVIII:C titrated calibrator plasma (i.e. C1). This solution has a FVIII:C titer of 200%. Prepare the following calibration curve by serial dilution in RUO 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>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII:C (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0%</td>
<td>200</td>
<td>100</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Volume of 200% FVIII:C Calibrator</td>
<td>500 µL</td>
<td>250 µL</td>
<td>125 µL</td>
<td>0 µL</td>
</tr>
<tr>
<td>Volume of RUO buffer</td>
<td>0 µL</td>
<td>250 µL</td>
<td>375 µL</td>
<td>500 µL</td>
</tr>
</tbody>
</table>

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.

RUO:
Reagent stability after reconstitution, free from any contamination or evaporation, and stored closed, is of:
• 72 hours at 2-8°C.
• 24 hours at room temperature (18-25°C).
• 3 months frozen at -20°C or less
• Stability on board of the analyzer: see the specific application.

RUO:
Reagent stability after reconstitution, free from any contamination or evaporation, and stored closed, is of:
• 3 months at 2-8°C.
• 7 days at room temperature (18-25°C).
• 3 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.

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.

If the substrate become 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 (and point method).
• Reference materials for FVIII:C assay in therapeutic concentrates (international or internal).
• Specific calibrators and controls with known titration, such as:

Product Name Reference
BIOPHEN™ Plasma Calibrator 222101-RUO
BIOPHEN™ Normal Control Plasma 223201-RUO
BIOPHEN™ Abnormal Control Plasma 223301-RUO

For low range calibration, dilute the calibrator in FVIII:C deficient plasma (DPP40A/K-RUO). Also refer to the specific application guide of the analyzer used.

WARRANTS 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 freez-drying stopper, to avoid any product loss when opening the vial.

RUO:
Reconstitute the contents of each vial with exactly:
REF 221402-RUO 1 mL of distilled water
REF 221406-RUO 2 mL of distilled water

Shake vigorously until complete dissolution (ensure that there is no deposit at the bottom of the glass) 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.

RUO:
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.
The calibration curve can also be established from a FVIII:C titrated reference material (international standard or internal standard).

Pre-dilute this material in \([R4]\) buffer to obtain a 1 kU/mL solution, then dilute 1:20 in \(R4\) to obtain a solution with a 200 FVIII:C unit/mL FVIII:C titer. Use this solution to establish a calibration curve in \([R4]\) buffer as previously explained.

**Low range (0 to 25%):**

Calibration can be performed using a pool of diluted normal plasmas, or a commercial calibrator plate with a known concentration of FVIII:C. The dilution factor in defective plasma is of 4 for the normal pool and of 4x100 for a calibrator with a concentration of C.

The assay method includes a 1:10 plasma dilution. The calibration curve ranges from 0 to 25% FVIII:C. The 1:10 dilution in \([R4]\) buffer represents 25% FVIII:C.

Using this solution, establish the following calibration curve in \([R4]\) buffer:

<table>
<thead>
<tr>
<th>Volume of 25% FVIII:C Calibrator</th>
<th>Specimens Reference Range</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 µL</td>
<td>High</td>
<td>1:40</td>
</tr>
<tr>
<td>12.5 µL</td>
<td>Low</td>
<td>1:10</td>
</tr>
<tr>
<td>25 µL</td>
<td>N.A.</td>
<td></td>
</tr>
</tbody>
</table>

Prepare the calibration curve immediately before use to avoid any FVIII:C degradation.

2. Dilute the specimens in \([R4]\) buffer, as described in the table below:

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Reference</th>
<th>Range</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen, calibrator or control diluted in ([R4])</td>
<td>High</td>
<td>1:40</td>
<td></td>
</tr>
<tr>
<td>Human factor X Pre-incubated at 37°C</td>
<td>Low</td>
<td>1:10</td>
<td></td>
</tr>
<tr>
<td>Activation Reagent Pre-incubated at 37°C</td>
<td>N.A.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mix and incubate at 37°C for 5 minutes, then add the following:

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid (2%)</td>
<td>50 µL</td>
<td>100 µL</td>
</tr>
<tr>
<td>R2</td>
<td>50 µL</td>
<td>100 µL</td>
</tr>
<tr>
<td>R4</td>
<td>50 µL</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

Mix and mix the optical density at 405nm against the corresponding blank.

**Kinetic method:**

The assay can be performed by the kinetic method by measuring the change in absorbance of 25°C, test the diluted specimens quickly. The exact calibrator and control concentrations for each batch are indicated on the analyzer provided with the kit.

Create a plasma blank if sample is icteric, lipaemic, haemolysed, or if its color differs from the standard plasma.

4. **Quality control:**

The use of quality controls serves to validate the 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 acceptable range for the method. Each laboratory must define its acceptance ranges and verify the expected performance in its analytical system.