**REAGENT PREPARATION AND STABILITY:**

The reagents are lyophilized under vacuum in their vials. To avoid any product loss when opening the vial of lyophilized reagents, gently remove the freeze-drying stopper.

**RT**

**Reagent 1: Thrombin reagent**

Brown vial. Reconstitute the contents of each vial with exactly 4 mL distilled water, shake vigorously until fully dissolved, while avoiding formation of foam. Allow to stabilize for 30 min. at room temperature (18-25°C), shaking occasionally.

Homogenize the reagent prior to use.

Reagent stability after reconstitution, excluding any contamination or evaporation, and stored in the original vial, is of:

- 1 week at 2-8°C.
- 48 hours at room temperature (18-25°C).
- 2 months frozen at -20°C or less.

**R2**

**Reagent 2: Detection reagent**

White vial. Reconstitute the contents of each vial with exactly 5 mL distilled water, shake vigorously until fully dissolved, while avoiding formation of foam. Allow to stabilize for 30 min. at room temperature (18-25°C), shaking occasionally.

Homogenize the reagent prior to use.

Reagent stability after reconstitution, excluding any contamination or evaporation, and stored in the original vial, is of:

- 1 week at 2-8°C.
- 48 hours at room temperature (18-25°C).
- 2 months frozen at -20°C or less

*Thaw only once, as rapidly as possible at 37°C, adapting the incubation period to the volume of reagent. The stability of the thawed reagent should be checked under laboratory work conditions.

**STORAGE CONDITIONS:**

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.

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

**Reagents:**

- Distilled water
- Physiological Saline (0.9% NaCl)
- Specific calibrators and controls with known FXIII titration, traceable to the International Standard for FXIII in plasma.

**Materials:**

- Automatic instrument for chromogenic assays with wavelength at 340 nm.
- Calibrated pipettes; Plastic tubes.

**SPECIMEN COLLECTION AND PREPARATION:**

Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5 guidelines for further information concerning specimen collection, handling and storage).

**Specimens:**

- Human plasma obtained from anticoagulated blood (trisodium citrate).
- Whole blood.
- Sodium citrated poor plasma.

**Collection:**

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M) by clean venipuncture. Discard the first tube.

**Centrifugation:**

Within 2 hours, use a laboratory-validated method to obtain platelet-poor plasma, for example at least 15 minutes at 2500g at room temperature (18-25°C) and allow the plasma to settle in a plastic tube.

**Plasma storage:**

- 8 hours at room temperature (18-25°C).
- 2 months at -20°C.
- 4 months at -70°C.

Frozen plasma specimens should be thawed rapidly at 37°C, then shaken thoroughly and tested immediately. Resuspend any precipitate by shaking vigorously immediately after thawing and before use.
**PROCEDURE:**
The kit can be used in kinetics mode on automated methods. Perform the test at 37°C and read the absorbance at 340nm.

**Automated methods:**
See the specific application and specific precautions for each analyzer (provided on request for various instruments according to availability; contact your local distributor for CS-series applications).

**Assay method:**
1. Reconstitute the calibrators and controls as indicated in the specific instructions. For preparing the calibration curve, dilute the calibrator in physiological saline to calibrate from approximately 0 to 150% FXIII. The 1:2 working dilution in physiological saline (in the schema below) corresponds by definition to 100% for a normal plasma pool, or C% FXIII for a commercial calibrator.

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>C</th>
<th>C:2</th>
<th>C:4</th>
<th>C:8</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume calibrator</td>
<td>500µL</td>
<td>250µL</td>
<td>125µL</td>
<td>60µL</td>
<td>0µL</td>
</tr>
<tr>
<td>Volume Physiological Saline</td>
<td>0µL</td>
<td>250µL</td>
<td>375µL</td>
<td>420µL</td>
<td>500µL</td>
</tr>
</tbody>
</table>

The point 3C:2 (or 150% for a normal plasma pool) is obtained by addition of 30 µL calibrator + 10 µL physiological saline in the table below.

2. Establish the calibration curve and test it with the quality controls. The exact calibrator and control concentrations for each batch are indicated on the flyer provided with the kit.

3. As an example, the here below table shows the schema for application on CS-series. Dispense the following to the reaction cuvettes incubated at 37°C (directly managed by the analyzer):

<table>
<thead>
<tr>
<th>Volume</th>
<th>Specimen, calibrator or control</th>
<th>20 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Physiological saline</td>
<td>20 µL</td>
</tr>
<tr>
<td></td>
<td>R1 Thrombin reagent, pre-incubated at 37°C</td>
<td>80 µL</td>
</tr>
<tr>
<td></td>
<td>Mix and incubate at 37°C for exactly 110 seconds, then add the following:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R2 Detection reagent, pre-incubated at 37°C</td>
<td>100 µL</td>
</tr>
<tr>
<td></td>
<td>Mix, incubate at 37°C, and measure (kinetics mode) the optical density (OD)/min at 340 nm between 200 and 500 seconds</td>
<td></td>
</tr>
</tbody>
</table>

If a reaction volume other than that specified above is required, it is recommended to use 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™ Factor XIII assay can be calibrated for the assay of FXIII activity in plasma.

Using a linear scale:
- The test is linear from 5 to 150% of FXIII on Sysmex CS-5100 (at the standard dilution).

The calibration curve shown below, obtained on Sysmex CS-5100 analyzer, is given as an example only. The calibration curve established for the assay series must be used.

**RESULTS:**
- On the Sysmex CS-series analyzer, the calibration curve is obtained in Lin-Lin scale, with the OD/min at 340 nm along the Y-axis and the FXIII concentration, expressed as %, along the X-axis. The concentration of Factor XIII in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.
- Results are expressed in percentage.
- The results should be interpreted according to the patient’s clinical and biological condition.

**LIMITATIONS:**
- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully. The laboratory is responsible for validating any changes made to these instructions for use.
- Any reagent presenting an unusual appearance or showing signs of contamination must be rejected.
- Any plasma displaying a coagulum or showing signs of contamination must be rejected.
- For the possible influence of interferences, refer to specific application for the analyzer used (no significant effect is observed on Sysmex CS-5100 for Heparin concentration up to 2 IU/mL, bilirubin concentration up to 60 mg/dL, hemoglobin concentration up to 250 mg/dL, fibrinogen concentrations up to 100 g/L, and fibrinogen concentrations from 0.8 up to 6 g/L by plasma overload tests. For high concentrations, an additional (eg 1:3) pre-dilution could be used and the result multiplied by the complementary dilution factor).

**EXPECTED VALUES:**
The reference range established on healthy adult subjects (n=120) using Sysmex CS-5100 (Central 90%, 95% percentile) was measured between 60 and 146 %. However, each laboratory has to determine its own normal range.

**PERFORMANCES:**
- The lower analyzer detection limit depends on the analytical system used (0.5% on Sysmex CS-5100).
- On Sysmex CS-series, the measuring range is from about 5 to 300% of FXIII.
- Performance studies were conducted internally on 1 batch of reagent using a Sysmex CS-5100. Performance was assessed using laboratory controls over a 5-day period, 2 series per day and triplicates within each series for a control level. The following results were obtained:

<table>
<thead>
<tr>
<th>Control</th>
<th>Intra assay</th>
<th>Inter assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Mean</td>
<td>CV%</td>
</tr>
<tr>
<td>Normal</td>
<td>40</td>
<td>102.3</td>
</tr>
<tr>
<td>Abnormal</td>
<td>40</td>
<td>28.8</td>
</tr>
</tbody>
</table>

**REFERENCES:**

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