HEMOCLOT™ Factor VIIa
Ref CK092K-RUO
R1, R2: 3 x 2 mL; R3: 3 x 20 mL

Clotting assay for the quantitative determination of FVIIa activity, in purified or plasmatic medium.

FOR RESEARCH USE ONLY.
DO NOT USE IN DIAGNOSTIC PROCEDURES

INTENDED USE:
The HEMOCLOT™ Factor VIIa kit is a clotting method for the quantitative determination of activated Factor VII (FVIIa) activity, in purified medium or citrated plasma, using manual or automated method. This kit is for research use only and must not be used for patient diagnosis or treatment.

PRINCIPLE:
FVIIa forms an enzymatic complex with recombinant truncated human Tissue Factor (rTF); this recombinant tissue factor protein does not promote Factor VII activation. Clotting is initiated by the addition of Calcium (Ca²⁺). Clotting time is then recorded. FVIIa being the limiting factor, there is a direct linear relationship between the FVIIa concentration and the corresponding clotting time.

REAGENTS:
R1: Factor VII deficient plasma: citrated human plasma, deficient for Factor VII, immuno-depleted, lyophilized in the presence of glycine and stabilizers. 3 vials of 2mL.
R2: Factor VIIa Cot-Pips: Human recombinant truncated TF (rTF) and synthetic Phospholipids, at the optimized concentration for the assay, lyophilized in presence of stabilizers. Contains BSA. 3 vials of 2mL.
R3: Heps BSA buffer: specific Heps-BSA dilution buffer, at pH 7.40. Ready to use. Contains BSA. 3 vials of 20mL.

Reagent R3 contains small amounts of sodium azide (0.9 g/L), see WARNINGS AND PRECAUTIONS.

WARNINGS AND PRECAUTIONS:
• Biological products must be handled with all necessary precautions and considered as being potentially 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. Do not mix reagents from different kit batches when performing an assay; they are optimized for each batch of kits.
• Handle the reagents with care to avoid contamination during use. If possible, avoid reagent evaporation during use by limiting the liquid-air exchange surface. Evaporation reduces the reagent's stability in the analyzer.
• To preserve reagent stability, seal the vials after use with their respective caps.
• Aging studies, conducted over a 3-week period at 30°C, show that the reagents can be shipped at room temperature over a short period of time, without degradation.
• The human plasma used to prepare the deficient plasma has been tested by recorded methods and is certified free of HIV antibodies, Hbs Antigen and HCV antibodies. The bovine plasma used to prepare the BSA has been tested by recorded methods and is certified free of infectious agents, in particular the causative agent of bovine spongiform encephalitis.
• For in vitro use.

REAGENT PREPARATION AND STABILITY:
The reagents are lyophilized under a vacuum in their vials. To avoid any product loss when opening the vial of lyophilized reagents, gently remove the freeze-drying stopper.
R1: Reagent 1: Factor VII deficient plasma
Reconstitute the contents of each vial with exactly 2 mL distilled water, shake vigorously until fully dissolved. 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:
• 3 days at 2-8°C.
• 48 hours at room temperature (18-25°C).
• 2 months frozen at -20°C or less

R2: Reagent 2: Factor VIIa Cot-Pips
Reconstitute the contents of each vial with exactly 2 mL distilled water, shake vigorously until fully dissolved. 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:
• 3 days 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.

R3: Reagent 3: Hepes-BSA buffer
Clear vial, ready to use. Allow to stabilize for 30 minutes at room temperature (18-25°C), before use. Homogenize the reagent prior to use.
Reagent stability after opening, excluding any contamination or evaporation, and stored in the original vial, is of:
• In its original vial, until the expiration date printed on the label, at 2-8°C.
• When open, 7 days at 2-8°C.

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.
• CaCl₂ 0.025M (AR001A/K).
• Specific Calibrators and controls with a known concentration, such as International Standard for FVIIa (NIBSC) or internal reference preparations and controls for FVIIa.
• Specific calibrators and controls with known titration, such as:

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIOPHeny™ Calibrator Factor VIIa</td>
<td>226301-RUO</td>
</tr>
<tr>
<td>BIOPHEN™ FVIIa Control Set 224901-RUO</td>
<td></td>
</tr>
</tbody>
</table>

Materials:
• Water-bath, semi-automatic or automatic instrument for clotting assays
• Stopwatch; Calibrated pipettes; Plastic tubes or microplates.

SPECIMEN COLLECTION AND PREPARATION:
Specimens should be prepared and stored in accordance with applicable local guidelines.
• Specimen:
Human plasma obtained from anticoagulated blood (trisodium citrate).
• 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-free plasma, for example at least 15 minutes at 2500 g at room temperature (18-25°C) and allow the plasma to settle in a plastic tube.
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 clotting time (sec) along the Y-axis and the FVIIa concentration, expressed as mIU/mL, along the X-axis.
• The concentration of FVIIa in the test specimen is directly inferred from the calibration curve, if the standard dilution is used. When complementary predilution are used, the measured FVIIa concentration must be multiplied by the complementary predilution factor to obtain the concentration in the tested plasma.
• Results are expressed in mIU/mL FVIIa.

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. 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 suspicious samples or those showing signs of activation must be rejected.
• Any plasma displaying a coagulum or showing signs of contamination must be rejected.

For samples measured > 500 mIU/mL, an additional 2 fold (or more) dilution can be used and obtained results multiplied by the additional dilution factor.

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 (UHF or LMWH) concentration up to 0.5 mIU/mL, bilirubin concentration up to 30 mg/dL, hemoglobin or intralipids concentrations up to 1000 mg/dL and Apixaban, Rivaroxaban or Dabigatran up to 50 ng/mL, by plasma overload tests).

PERFORMANCE:
• The lower analyzer detection limit on Sysmex® CS-5100 is <1 mIU/mL.
• The assay working range is from 5 to 500 mIU/mL.
• HEMOCLOT™ Factor VIIa assay is insensitive to FVII at normal concentration.
• Performance studies were conducted internally on 3 batches of reagent using a Sysmex® CS-5100. Performance was assessed using laboratory controls, from 40 values (within run) or for between run from a 20-day period, 2 series per day and 3 replicates within each series for a control level. The following results were obtained:

<table>
<thead>
<tr>
<th>Control</th>
<th>n</th>
<th>Mean (mIU/mL)</th>
<th>CV%</th>
<th>SD</th>
<th>n</th>
<th>Mean (mIU/mL)</th>
<th>CV%</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>QC1</td>
<td>120</td>
<td>83.7</td>
<td>4.3</td>
<td>3.6</td>
<td>120</td>
<td>83.7</td>
<td>4.3</td>
<td>3.6</td>
</tr>
<tr>
<td>QC2</td>
<td>120</td>
<td>265.9</td>
<td>2.7</td>
<td>7.2</td>
<td>120</td>
<td>265.9</td>
<td>2.7</td>
<td>7.2</td>
</tr>
</tbody>
</table>

REFERENCES:

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

Changes compared to the previous version.