HEMOCLOT™ Factor VIIa

Ref CK092K

R1, R2: 3 x 2 mL; R3: 3 x 20 mL

IVD

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

Not for Sale in the US

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INTENDED USE:

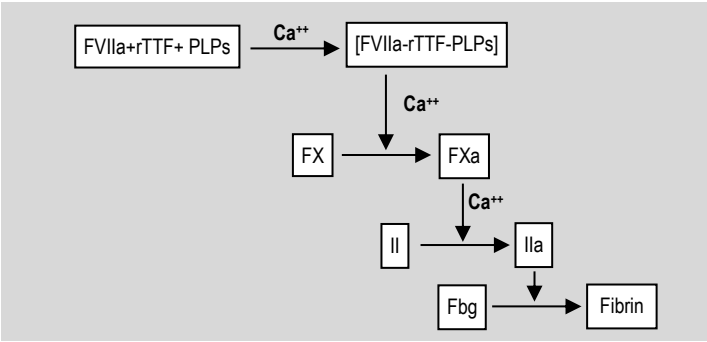
The HEMOCLOT™ Factor VIIa kit is a clotting method for the quantitative determination of activated Factor VII (FVIIa) activity, in purified medium or citrated plasma, using manual or automated method.

SUMMARY AND EXPLANATION:

FVIIa is a serine esterase of the extrinsic coagulation pathway[1]. When complexed to Tissue Factor (TF), in presence of phospholipids and Calcium, it activates Factor X to Factor Xa.
HEMOCLOT™ Factor VIIa is a clotting assay for testing FVIIa activity, it is insensitive to Factor VII.

PRINCIPLE:

FVIIa forms an enzymatic complex with recombinant truncated human Tissue Factor (rTTF); this recombinant tissue factor protein does not promote Factor VII activation[2]. 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 Cof-Plps: Human recombinant truncated TF (rTTF) and synthetic Phospholipids, at the optimized concentration for the assay, lyophilised in presence of stabilizers. Contains BSA.
3 vials of 2mL.

R3: Hepes BSA buffer: specific Hepes-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-weeks 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* diagnostic 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 Cof-Plps
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.
- $CaCl_2$ 0.025M (AR001A/K).
- Specific Calibrators and controls with a known concentration, such as International Standard for FVIIa (NIBSC)[3] or internal reference preparations and controls for FVIIa.
- Specific calibrators and controls with known titration, such as:

Product Name	Reference
BIOPHEN™ Calibrator Factor VIIa	226301
BIOPHEN™ FVIIa Control Set	224901

Materials:

- Water-bath, semi-automatic or automatic instrument for clotting assays
- Stopwatch; Calibrated pipettes; Plastic tubes or microplate.

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[4] guidelines for further information concerning specimen collection, handling and storage).

- Specimens:

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-poor 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.

- Plasma storage:
  - 4 hours at room temperature (18-25°C).
  - 1 month at -20°C.
  - 18 months at -70°C[5].

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 is a clotting method, automated or manual (endpoint) methods. Perform the test at **37°C** and the clotting time, triggered by addition of Calcium Chloride, is measured.

Automated methods:

Applications for the various analyzers are available on request. **See the specific application and specific precautions for each analyzer.**

Assay method:

1. Reconstitute the calibrators and controls (2 levels recommended at about 75 and 250 mIU/mL), as indicated in the specific instructions or according to internal procedure.
Prepare calibration points in the range 12.5-500 mIU/mL. Dilute them **1:10** in **R3** buffer for the test.

2. Dilute the specimens and controls in R3 buffer, as described in the table below:

Sample	Predilution	Dilution
Control	**No**	**1:10**
Specimen (plasma)	**No**	**1:10**
Specimen (FVIIa in purified medium)	**Adjusted to 25-400 mIU/mL optimal range, in R3 buffer**	**1:10**

Establish the calibration curve and test it with the quality controls. If stored at room temperature (18-25°C), test the diluted specimen within 1 hour. The exact calibrator and control concentrations for each batch are indicated on the flyer provided with the kit.

For information, correspondence between ng/mL and IU/mL:

Concentration in ng/mL	Concentration in IU/mL
20 ng/mL	1 IU/mL
1 ng/mL	50 mIU/mL

3.

Reagents	Volume
Calibrators, Controls or tested specimen (1:10 diluted in R3)	50 µL
R1: FVII Deficient Plasma. Preincubated at 37°C	50 µL
Mix and Incubate **at 37°C** for **1 minute**, then introduce:	
R2 : rTTF + Phospholipids. Preincubated at 37°C	50 µL
Mix and Incubate **at 37°C** for **2 minutes**, then introduce:	
CaCl2 0.025M (preincubated at 37°C, and stirred)	50 µL
Record Clotting Times	**CT (sec)**

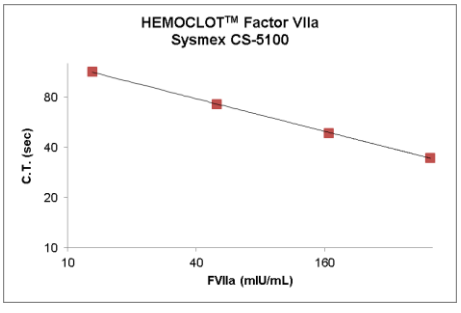
If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

CALIBRATION:

The HEMOCLOT™ Factor VIIa assay can be calibrated for the assay of FVIIa in plasma or purified medium.
The 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.
Using a bi-logarithmic scale:

- The test is linear up to at least 500 mIU/mL (up to 1000 mIU/mL on Sysmex® CS series and STA-R®).

The calibration curve shown below, obtained with the international standard NIBSC for FVIIa Concentrate, on Sysmex® CS-5100 analyzer, is 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 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 sample.
- Results are expressed in mIU/mL FVIIa.
- On plasma, 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 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 (UFH or LMWH) concentration up to 0.5 IU/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).

EXPECTED VALUES:

FVIIa therapeutic range[6] should be defined according to the current local recommendations. Healthy individuals values vary from laboratory to laboratory, each laboratory should determine its own normal range.
In a study with ostensibly healthy subjects (n = 120) on Sysmex® CS-5100, the following reference interval (Central 90%, 95th percentile) was determined: 20 - 114 mIU/mL.

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:

Control	Intra assay				Inter assay			
	n	Mean (mIU/mL)	CV%	SD	n	Mean (mIU/mL)	CV%	SD
QC1	40	82.7	1.9	1.6	120	83.7	4.3	3.6
QC2	40	261.5	2.2	3.6	120	262.9	2.7	7.2

REFERENCES:

1. Giansily-Blaizot M, *et al,*. Study group of FVII deficiency. Analysis of biological phenotypes from 42 patients with inherited factor VII deficiency: can biological tests predict the bleeding risk? Haematologica. 2004
2. Neuenschwander PF, *et al,*. Deletion of the membrane anchoring region of tissue factor abolishes autoactivation of factor VII but not cofactor function. Analysis of a mutant with a selective deficiency in activity.J Biol Chem. 1992
3. WHO International Standard, Blood Coagulation Factor VIIa, Concentrate, Human, 2nd International Standard, NIBSC 07/228.
4. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008
5. Woodhams B, *et al,*. Stability of coagulation proteins in frozen plasma. Blood coagulation and Fibrinolysis. 2001.
6. Logan AC and Goodnough LT. Recombinant factor VIIa: an assessment of evidence regarding its efficacy and safety in the off-label setting. Hematology. 2010.

SYMBOLS:

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

Changes compared to the previous version.