HYPHEN BioMed
www.hyphen-biomed.com
155, rue d'Eragny
95000 NEUVILLE SUR OISE
FRANCE
Tel. : +33 (0)1 34 40 65 10
Fax : +33 (0)1 34 48 72 36
info@hyphen-biomed.com

CE
IVD

HEMOCLOT™ Factor VIIa

Ref CK092K (3 x 2 mL)

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

Not for Sale in the US

English, last revision: 09-2016

INTENDED USE:

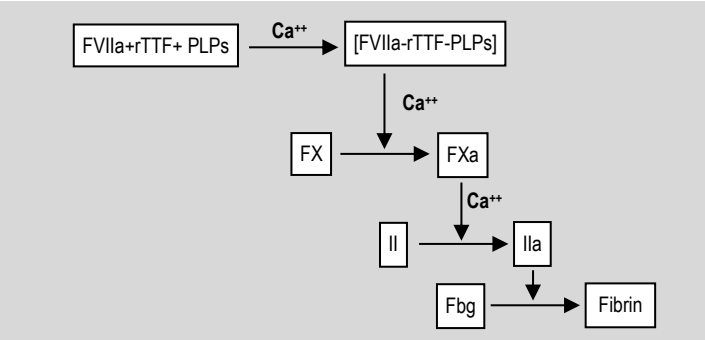
The HEMOCLOT™ Factor VIIa kit is a clotting assay for the quantitative determination of activated factor VII (FVIIa) activity, in purified medium or citrated plasma, using manual or automated method.

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.

ASSAY 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: Reagent 1: 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: Reagent 2: Factor VIIa Cof-Plps: Human recombinant truncated TF (rTTF) and synthetic Phospholipids, at the optimized concentration for the assay, lyophilised in presence of stabilizers.
3 vials of 2 mL.
R3: Reagent 3: Hepes BSA buffer: specific Hepes-BSA dilution buffer, at pH 7.40. Ready to use.
3 vials of 20 mL.
Reagent R3 contains low concentration of Sodium azide (0.9 g/L), see CAUTIONS AND WARNINGS

CAUTIONS AND WARNINGS:

- Any product of biological origin must be handled carefully, as being potentially infectious.
- Sodium azide can generate explosive components in contact with lead or copper pipes.
- The disposal of waste materials must be carried out according to current local regulations
- Use only reagents from kits with the same lot number. Do not mix reagents from kits with different lots when running the assay; they are optimized for each lot of kits.
- Reagents must be handled with care, in order to avoid any contamination during use. Take care to limit as much as possible any evaporation of the reagents during use, by limiting the liquid-air surface exchange. Evaporation reduces reagent stability on instrument board.
- In order to preserve the stability of the reagents, close the vials with their original screw cap following each use.
- Stability studies for 3 weeks at 30°C show that the reagents can be shipped at room temperature for a short period without damage.
- For in vitro diagnostic use.
- Bovine plasma used for the Bovine Serum Albumin (BSA) preparation was tested and certified free from infectious diseases, including Bovine Spongiform Encephalopathy (BSE). Human plasma used for the deficient plasma preparation was tested by registered methods with negative results for at least HIV antibodies, HBs Ag and HVC antibodies.

PREPARATION AND STABILITY OF REAGENTS:

Vials are closed under vacuum. Remove carefully the stopper, in order to avoid any loss of powder when opening the vials.

R1: Reagent 1: Factor VII deficient plasma
Reconstitute each vial with exactly **2 mL** of distilled water, shake thoroughly for complete dissolution, let the reagent stabilize for 30 min at room temperature (18-25°C); while shaking the vial from time to time.
Homogenize the reagent before each use.
Stability of reagent after reconstitution, provided that any contamination or evaporation is avoided, kept in its original vial:
- **3 days** at 2-8°C.
- **48 hours** at room temperature (18-25 °C).
- **2 months** at -20°C or below*

R2: Reagent 2: Factor VIIa Cof-Plps
Reconstitute each vial with exactly **2 mL** of distilled water, shake thoroughly for complete dissolution, let the reagent stabilize for 30 min at room temperature (18-25°C); while shaking the vial from time to time.
Homogenize the reagent before each use.
Stability of reagent after reconstitution, provided that any contamination or evaporation is avoided, kept in its original vial:
- **3 days** at 2-8°C.
- **48 hours** at room temperature (18-25 °C).
- **2 months** at -20°C or below*

*Thaw once as rapidly as possible at 37°C, adapt incubation duration to the volume of reagent. The stability of the thawed reagent should be verified in the working conditions of the user laboratory.

R3: Reagent 3: Hepes-BSA buffer
Clear vial, ready to use buffer. Let the reagent stabilize for 30 minutes at room temperature (18-25°C), before use.
Homogenize before each use.
Stability of reagent provided that any contamination or evaporation is avoided:
- 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 must be stored at 2-8°C, in their original packaging box. They are then usable until the expiration date printed on the label.

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED:

Reagents:
- Distilled water
- $CaCl_2$ 0.025 M *(reference AR001A/AR001K)*
- Specific Calibrators and controls with a known concentration, such as International Standard for FVIIa (NIBSC)[3] or internal reference preparations and controls for FVIIa.

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

SPECIMEN COLLECTION:

Preparation and storage of specimens must be performed according to the current local regulations (In the USA, refer to CLSI Document H21-A5 for further instructions on specimen collection, handling and storage[4]).

- Specimens:

Human plasma obtained from trisodium citrate anticoagulated blood.

- Collection:

Blood (9 vol.) must be collected on trisodium citrate anticoagulant (1 vol.) (0.109M), with caution, through a net venipuncture in order to avoid any activation. The first tube must be discarded.

- Centrifugation:

Within 2 hours, use a validated method in the laboratory to obtain a platelet-poor plasma, e.g., a minimum of 15 minutes at 2500 g at room temperature (18-25°C) and plasma must be decanted into a plastic tube.

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

Frozen plasma specimens should be rapidly thawed at 37°C, then gently mixed and tested immediately. Resuspend any precipitation by thorough mixing immediately after thawing and before testing.

TEST PROCEDURE:

The HEMOCLOT™ Factor VIIa kit is a clotting method, manual or automated. The assay is performed **at 37°C**, and the clotting time, triggered by addition of Calcium chloride, is measured.

Automated methods:

Applications to the various analyzers are available upon request. **Refer to each specific applications and specific cautions for each instrument.**

Assay method:

1. Reconstitute the reference preparation or calibrator, and controls (2 levels recommended at about 75 and 250 mIU/mL), using the specific package inserts 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 samples and controls using 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**

Run the calibration curve and test it with quality controls. Diluted sample should be tested within 1 hour when stored at room temperature (18-25°C).

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)**

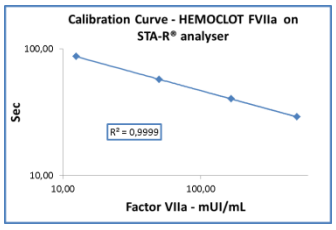
If an other reactive mixture volume than this indicated here above is required for the method used, the volumes ratio must be strictly respected, in order to assure the assay performances. It is responsibility of the user to validate any modifications and their impact on all assay results.

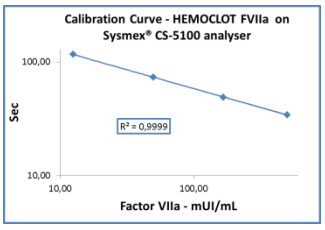
CALIBRATION:

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

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

The calibration curves below, obtained with the international standard NIBSC for FVIIa Concentrate, on STA-R® or Sysmex® CS-5100, are indicated as an example only. The calibration curve generated for the series of measures performed must be used.





QUALITY CONTROL:

Using quality controls, allows validating the calibration curve, as well as the homogeneous reactivity from run to run, when using a same lot of reagents.
Quality control must be included in each series, as per good laboratory practice, in order to validate test results. A new calibration curve must be carried out preferentially for each test series, and at least for each new lot of reagents or, after each important analyzer's maintenance, or when quality controls values are measured outside the acceptance range determined for the method.
Each laboratory should establish and verify its own target values, acceptance ranges and expected performances, according to the instruments and protocols used.

RESULTS:

- For the manual method, draw the calibration curve on a bi-logarithmic graph paper plot, with on abscissae, the FVIIa concentration (mIU/mL) and on ordinates the corresponding clotting times (CT, sec).
- The FVIIa concentration in the tested specimen is directly deduced from the calibration curve. When predilutions are used, multiply the measured FVIIa concentration by the predilution factor in order to get the concentration in the tested specimen.
- Results are expressed in FVIIa activity (mIU/mL).
- On plasma, the results are to be interpreted according to the patient's clinical and biological states.

LIMITATIONS:

- In order to get the optimal performances of the assay, the technical instructions must be strictly respected.
- Any reagent presenting an unusual aspect or contamination signs must be rejected.
- Any plasma containing a coagulum or contamination signs 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.

EXPECTED VALUE:

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.

PERFORMANCES:

- The lower limit of detection on Sysmex® CS-5100 is <1 mIU/mL.
- The assay working range is 5 to 500 mIU/mL
- HEMOCLOT™ Factor VIIa assay is insensitive to FVII at normal concentration. Test results are not affected by Heparin (UFH or LMWH) up to 0.5 IU/mL, Haemoglobin or Intralipid up to 1000 mg/dL, Bilirubin up to 30 mg/dL, and Apixaban, Rivaroxaban or Dabigatran up to 50 ng/mL.
- Performance study was performed in-house using 3 lots of reagent on Sysmex® CS-5100. Performances were evaluated with quality controls obtained from diluted International Standard NIBSC for FVIIa, from 40 values (within run) or for between run from 20 days, 2 runs per day and 3 replicates per run for each control level. Following data were obtained:

Control	Within-Run				Between-run			
	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:

Used symbols and signs listed in the ISO standard 15223-1.