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CE

HEMOCLOT FVII reagent

 # CK081K

Measurement of Factor VII activity with a clotting method

For in vitro diagnostic use only Not for Sale in the US

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1. Intended use:

The **HEMOCLOT FVII reagent** kit is proposed for the measurement of Factor VII clotting activity in human citrated plasma using a clotting method, triggered with calcium thromboplastin.

2. Assay principle:

The **HEMOCLOT FVII reagent** method is a clotting assay where all the extrinsic pathway clotting factors are present and in excess, excepted for factor VII, which is brought by the diluted tested plasma, and thromboplastin.
Factor VII is the limiting factor and clotting time is inversely proportional to the factor VII concentration. There is an inverse linear relationship, on a bilogarithmic graph paper, between the factor VII concentration and the corresponding clotting time.

3. Assay specimen:

Human plasma prepared from Trisodium Citrate anticoagulated blood.

4. Reagents:

Each kit contains 6 vials of 1 ml of **HEMOCLOT FVII (Def.) reagent**, a clotting mixture containing purified bovine Fibrinogen and Factor V, human Prothrombin and Factor X, lyophilized in presence of preservatives and stabilizers.

5. Reagents and material required, but not supplied:

- Pipettes with dispensing volumes of 20 µl, 50 µl and 100 µl.
- Pipette with a variable dispensing volume from 50 µl to 1,000 µl.
- Semi-automatic or automatic coagulation instrument, or fibrometer or electromagnetic water bath.
- Distilled water.
- Imidazole buffer (# AR021A/AR021K/AR021L).
- Normal human plasma pool or Factor VII calibrator (BIOPHEN Plasma Calibrator - # 222101).
- Normal and Abnormal control plasmas, titrated for factor VII, (BIOPHEN Normal Control Plasma - #223201 and BIOPHEN Abnormal Control Plasma - #223301).
- Calcium Thromboplastin (such as rabbit brain thromboplastin).

6. Reagent preparation and stability:

In the original package, and before any use, when stored at 2-8°C, the **HEMOCLOT FVII reagent** is stable until the expiration date printed on the kit.

Note: The stability studies at 30°C show that all the reagents can be shipped at room temperature without damage.

- **Reagent Preparation**:

Restore the vial with **1 ml** of distilled water; mix gently until complete dissolution of the content (vortex), let for 15 min. at room temperature(18-25°C); homogenize before each use.

- **Reagent stability following reconstitution:**
  - 24 hours at room temperature (18-25°C).
  - 72 hours at 2-8°C.
  - 1 month frozen at -20° or below.

Note:
In order to improve stability, reagents must be closed with their original screw cap following each use.
Reagents must be handled with care, in order to avoid any contamination during use.
Vials are closed under vacuum. Remove carefully the stopper, in order to avoid any lost of powder when opening the vials.
Proteins were prepared from human plasma, which was tested with registered methods and found negative for HIV antibodies, HBs Ag and HVC antibodies. Bovine Serum Albumin (BSA) and proteins were prepared from bovine plasma, which was tested for the absence of infectious agents, and collected from animals free from BSE. However, no assay may warrant the total absence of infectious agents. Any product of biological origin must then be handled with all the required cautions, as being potentially infectious.

7. Sample collection and preparation:

Blood (9 vol.) must be collected on 0.109M trisodium citrate anticoagulant (1 vol.); plasma supernatant is decanted following a 15 min. centrifugation at 2,500 g; citrated plasma must be tested within 4 hours when stored at room temperature (18-25°C), or can be rapidly frozen at –20°C or below for up to 1 month. Just before use, the plasma must be thawed for 15 min. in a water bath at 37°C.

Refer to GEHT or NCCLS guidelines for further instructions on specimen collection, handling and storage. Discard any sample with an unusual aspect.

8. Protocol:

- **Calibration curve:**

Prepare 2 ml of normal citrated human plasma pool **diluted 1:10** in Imidazole buffer. By definition, this ten fold dilution of the normal citrated human plasma pool corresponds to a concentration of **100% of factor VII.** Using this preparation, the calibration curve is obtained as follows:

VII	6.25%	12.5%	25%	50%	100%
Dilution	1:160	1:80	1:40	1:20	1:10
Plasma pool 1:10	0.060 mL	0.125 mL	0.250 mL	0.500 mL	1 mL
Imidazole Buffer	0.900 mL	0.875 mL	0.750 mL	0.500 mL	0 mL

The calibration curve can also be established with the BIOPHEN Plasma Calibrator (#222101), using the factor VII activity indicated on the flyer for the lot used.

The calibration curve must be used within 2 hours at room temperature.

- **Preparation of tested plasma:**

Tested plasma must be **diluted 1:10** in Imidazole buffer. The diluted plasma must be tested within 2 hours.

Caution : to ensure optimal performances of the assay, perform all assays (calibration, samples, controls) extemporaneously and successively without interruption

- **Assay:**

Manual Method:

Preincubate Calcium Thromboplastin at 37°C.

In a test tube, or a cuvette, introduce:

- 100 µl of **HEMOCLOT FVII reagent**.
- 100 µL of calibration solution or of tested plasma diluted **1:10**.

Incubate for 1 min. at 37°C, and then introduce (starting the stop watch):

- 150 µl of Calcium Thromboplastin preincubated at 37°C.

Record the clotting time.

Automatic Method:

The assay can be used with the semi-automatic or automatic instruments, such as STA-R, KC-4, KC-10, etc…

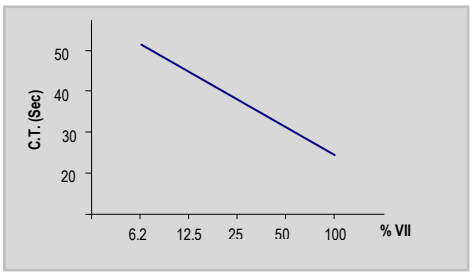
The usual program used for testing the factors involved in the extrinsic pathway with a clotting based calcium thromboplastin method, and a specific deficient plasma, can be applied. The respective specimen and reagent volume ratios indicated for the manual method must be strictly respected. Usually, with automatic methods the volumes used for reagents and tested plasma (diluted 1:10) are half those recommended for the normal method.

With semi automatic or automatic instruments, especially those with a photometric detection of clot formation, clotting times use to be slightly shorter than with the manual method.

9. Expression of results:

On a bilogarithmic graph paper, plot on abscissa the FVII concentrations and on ordinates the corresponding clotting times. On the calibration curve obtained, interpolate directly the corresponding VII concentration for the tested plasma.

Example of Calibration curve: This calibration curve is indicated as an example only. It was obtained with Neoplastin CI Plus from Diagnostica Stago, using a manual method.



10. Quality Control:

Use of quality control plasmas allows validating the calibration curve, as well as the homogeneous reactivity of the assay from run to run, and from series to series, when using a same lot of reagents. Various control plasmas are available:
BIOPHEN Normal Control Plasma: (ref 223201);
BIOPHEN Abnormal Control Plasma: (ref 223301).
Each laboratory should verify and validate its own target value and acceptance range, for each new lot of quality control used, according to the instrument used and in the laboratory working conditions.

11. Cautions and limitations:

The **HEMOCLOT FVII reagent does not contain heparin inhibitors**. Presence of heparin or of other anti-thrombin or anti-Xa substances may interfere in the assay and prolong the clotting time.

However, current Calcium Thromboplastin preparations use to contain an heparin inhibitor. The assay is then insensitive to the presence of heparin. If any risk of interference of heparin must be avoided, check that the Calcium Thromboplastin used contains an heparin inhibitor. However, heparin inhibitors, present in thromboplastin preparations, are not always totally efficient for neutralizing Low Molecular Weight Heparin (LMWH).
Sampling must be performed with great care, avoiding any blood activation. Discard any plasma presenting an unusual aspect, or any sign of activation or clotting.
It is recommended to perform all assays of fresh calibration points, specimen and controls successively without interruption, to obtain optimal performances of the assay.
For a better accuracy, samples measured ≤10% can be tested at the 1:5 dilution, and obtained results divided by 2; for samples measured >100% (or C%), the 1:20 dilution can be used and obtained results multiplied by 2.
For a deficient sample: check the result by testing if necessary the 1:5 dilution (the obtained concentration must then be divided by 2), and/or another sample and/or method for the patient plasma; check potential associated factor(s) deficiency.
Thrombin inhibitors present in the tested sample may lead to an underestimation of the FVII concentration
Do not store the plasma at 2-8°C.

12. Normal values:

Normal values for factor VII activity are usually > 60%.

13. Applications:

The **Hemoclot FVII reagent** is proposed for measuring factor VII in any clinical situation where it can be deficient.
The major applications are:

- Vitamin K deficiency (hepatic diseases, primary biliary cirrhosis, deficiency in new-borns, antibiotherapy, …)
- Vitamin K antagonists (dicoumarol therapy, …)
- Isolated deficiencies of factor VII.
- Accelerated clotting factor consumption (DIC)

14. Assay variations:

The clotting times observed for this assay are obtained with Calcium Thromboplastin from Diagnostica Stago (Neoplastin CI Plus). They are in the range from 25 to 35 seconds for the 100% VII concentration. Clotting times can vary according to the type of thromboplastin used. The assay performances can slightly vary according to the thromboplastin reagent type and lot, and the instrument used in the laboratory. Performances, as well as target values and acceptance ranges for each new lot of quality controls used, must then be confirmed (and adjusted if necessary) in the laboratory working conditions.

15. References:

1. Soulier JP, Larrieu MJ. Etude analytique des temps de Quick allongés. Dosage de prothrombine, de proconvertine et de proaccélérine. Sang 1952 ; 23: 549-559.
2. Favre-Gilly J, Belleville J, Croizat P, Revel L. Les états hémorragiques acquis par trouble plasmatique de coagulation. Cah Med Lyonnais 1967 ; 43 (28) : 2611-2668.
3. Gjonnaess H, Fagerhol MK. Studies on coagulation and fibrinolysis in pregnancy. Acta Obste Gynecol Scand 1975; 54: 363-367.
4. Andrew M, Paes B, Milner R, Hohnston M, Mithell L, Tollefsen DM, Powers P. Development of the human coagulation system in the full-term infant. Blood 1987; 70: 165-172.