HEMOCLOT™ VII+X

REF CK051K-RUO R 6 x 1 mL

REF CK051L-RUO R 20 x 1 mL

Clotting method for the determination of Factor VII+X activity.

FOR RESEARCH USE ONLY.

DO NOT USE IN DIAGNOSTIC PROCEDURES.

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English, last revision: 04-2021

INTENDED USE:

The HEMOCLOT™ VII+X kit is a clotting method, using calcium thromboplastin, for the *in vitro* quantitative determination of Factor VII+X (FVII+X) activity in human citrated plasma, using a manual or automated method.

This kit is for research use only and must not be used for patient diagnosis or treatment.

SUMMARY AND EXPLANATION:

Technical:

FVII and FX are glycoproteins, vitamin K-dependent, synthesized by the liver, belonging to the factors of the prothrombin complex: II, V, VII, X, Protein C, Protein S, Protein Z.

FVII is the serine esterase of the extrinsic coagulation pathway. In the Tissue Factor complex (FT), in the presence of phospholipids and calcium, activates factor X in factor Xa.

FX can be activated by the intrinsic or extrinsic pathways and initiates the common pathway of coagulation. In the presence of calcium and phospholipids, and in combination with factor Va, factor Xa forms a complex able to activate prothrombin thrombin.

PRINCIPLE:

The HEMOCLOT™ VII+X method is a clotting assay where all the extrinsic pathway clotting factors are present and in excess, excepted FVII and FX, which is brought by the diluted tested plasma, and thromboplastin.

FVII+X are the limiting factors and clotting time obtained is inversely proportional to the concentration of FVII+X. There is an inverse linear relationship, on a bi-logarithmic graph paper, between the FVII+X concentration and the corresponding clotting time.

REAGENTS:

R **HEMOCLOT™ VII+X:** Clotting reagent containing highly purified bovine prothrombin, fibrinogen and bovine Factor V, lyophilized in presence of preservatives and stabilizers. Contains BSA.

REF CK051K-RUO ➔ 6 vials of 1 mL.

REF CK051L-RUO ➔ 20 vials of 1 mL.

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of animal origin. Users of reagents of these types must exercise extreme care in full compliance with safety precautions in the manipulation of these biological materials as if they were infectious.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.
- Aging studies show that the reagents can be shipped at room temperature without degradation.
- This device of *in vitro* use is intended for professional use in the laboratory.

REAGENT PREPARATION:

Gently remove the freeze-drying stopper, to avoid any product loss when opening the vial.

R Reconstitute the contents of each vial with exactly **1 mL of distilled water.**

Shake vigorously until complete dissolution while avoiding formation of foam and load it directly on the analyzer following application guide instruction.

For manual method, allow to stabilize for 15 minutes at room temperature (18-25°C), homogenize before use.

STORAGE AND STABILITY:

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.

R Reagent stability after reconstitution, free from any contamination or evaporation, and stored closed, is of:

- **72 hours** at 2-8°C.
- **24 hours** at room temperature (18-25°C).
- **1 month** frozen at -20°C or less*
- **Stability on board of the analyzer: see the specific application.**

*Thaw only once, as rapidly as possible at 37°C and use immediately.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- Imidazole buffer (AR021B-RUO/AR021K-RUO/AR021L-RUO/ AR021M-RUO/AR021N-RUO).
- Calcium Thromboplastin.
- Specific calibrators and controls:

Product Name	Reference
BIOPHEN™ Plasma Calibrator	222101-RUO
BIOPHEN™ Normal Control Plasma	223201-RUO
BIOPHEN™ Abnormal Control Plasma	223301-RUO

Also refer to the specific application guide of the analyzer used.

Materials:

- Water-bath, semi-automatic or automatic analyzer for clotting assays.
- Stopwatch; Calibrated pipettes; silicon glass or plastic test tubes.

SPECIMEN COLLECTION AND PREPARATION:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture. Discard the first tube.

Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5[1] guideline for further information concerning specimen collection, handling and storage).

For plasma storage, please refer to references[1].

PROCEDURE:

The kit can be used in manual or automated method. Perform the test at 37°C and the clotting time, triggered by addition of Calcium Thromboplastin, is measured.

Assay method:

1. Reconstitute the calibrators and controls as indicated in the specific instructions. Prepare 2 mL of normal citrated human pooled plasma **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 FVII+X.** Use 1:10 dilution to prepare the following calibration curve:

Dilution	0	1:80	1:40	1:20	1:10
FVII+X (%)	0	12.5	25	50	100
Plasma pool 1:10	0mL	0.125mL	0.250mL	0.500mL	1mL
Imidazole Buffer	0mL	0.875mL	0.750mL	0.500mL	0mL

The calibration curve can also be established with the BIOPHEN™ Plasma Calibrator (222101-RUO), using the FVII+X activity (C) indicated on the flyer for the lot used. The calibration curve must be prepared just before running the assay.

2. Tested plasmas and controls must be diluted with imidazole buffer as described in the table below :

Specimens	Reference	Dilution
Control	223201-RUO / 223301-RUO	**1:10**
Specimens	N.A.	**1:10**

3. Dispense the following to the test tube or cuvette (procedure with NeoPTimal Stago):

	Volume
Calibrator, or diluted plasma or controls diluted **1:10**	75 µL
R **HEMOCLOT™ VII+X**	75 µL
Incubate **at 37°C for 1 or 2 minutes,** then add the following (starting the stopwatch) :	
NeoPTimal Stago preincubated at **37°C**.	50 µL
Record the exact clotting time (sec)	

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

The user is responsible for validating any changes and their impact on all results.

For an automated method, application guides are available on request. See specific application guide and specific precautions for each analyzer.

CALIBRATION:

The HEMOCLOT™ VII+X can be calibrated for the FVII+X assay. The plasma 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.

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 established, 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 acceptance range for the method.
Each laboratory must define its acceptance 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 FVII+X concentration, expressed as %, along the X-axis.
- The concentration of FVII+X (%) in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.

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.
- 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.

REFERENCES:

1. CLSI Document H21-A5: “Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline”. 2008.

SYMBOLS:

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

Changes compared to the previous version.