Deficient Plasma for Factors II, V, VII, X

CE
IVD

REF DP010A / K DP 1 x 1 mL / 6 x 1 mL
REF DP020A / K DP 1 x 1 mL / 6 x 1 mL
REF DP030A / K DP 1 x 1 mL / 6 x 1 mL
REF DP060A / K DP 1 x 1 mL / 6 x 1 mL

Deficient plasma for factors II, V, VII and X assay by clotting assay.

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INTENDED USE:

The Prothrombin Deficient Plasma, Factor V Deficient Plasma, Factor VII Deficient plasma and Factor X Deficient Plasma kits are respectively proposed for the quantitative determination of Factor II (FII or Prothrombin), of Factor V (FV or Proaccelerin), of Factor VII (FVII or Proconvertin) or of Factor X (FX or Factor Stuart) activity in human citrated plasma using a clotting method and calcium thromboplastin, via manual or automated method.

SUMMARY AND EXPLANATION:

Technical[1]:

The extrinsic pathway is triggered by tissue factor (TF), phospholipids, and calcium which allows to activate FVII (FVIIa). TF-FVIIa complex activates FX into FXa, which converts prothrombin to thrombin, in presence of its cofactor (activated FV).

Clinical[1,2,3]:

Factor VII deficiency is a rare bleeding disorder, symptomatic patients are usually homozygous, but bleeding is sometimes manifested in heterozygotes. The molecular pathology of the deficiency does not always correlate with the clinical manifestations in the patients. FX deficiency is extremely rare, severe homozygous FX deficiency may result in a clinically significant bleeding disorder. Patients with homozygous factor V deficiency have severe bleeding diathesis; sometimes heterozygotes in whom levels of factor V may be below the normal range have a bleeding tendency. Prothrombin deficiency is a rare hereditary coagulation disorder. Affected individuals may have a quantitative deficiency of prothrombin or may inherit an abnormal molecular variant of prothrombin. Congenital or acquired deficiencies of these factors exhibit a prolonged prothrombin time (PT).

PRINCIPLE:

The technique proposed is based on a clotting method where all the clotting factors are present (constant and in excess, brought by the deficient plasma), excepted for factor to measure, which is brought by the diluted tested plasma, and the clotting is triggered with calcium thromboplastin. Factor to measure is the limiting factor and clotting time is inversely proportional to the concentration of factor to measure. There is an inverse linear relationship, on a bilogarythmic graph paper, between the concentration of factor to measure and the corresponding clotting time.

REAGENTS:

DP **Citrated human plasma, deficient for Factor to measure (FII, FV, FVII or FX)**, lyophilized in the presence of glycine and stabilizers. This plasma is deficient for factors to measure (FII, FV, FVII or FX) (<1%), whereas all the other coagulation factors are within about the normal range (> 50%).

Prothrombin Deficient Plasma
REF **DP010A ➔ 1 vial of 1 mL.**
REF **DP010K ➔ 6 vials of 1 mL.**

Factor V Deficient Plasma
REF **DP020A ➔ 1 vial of 1 mL.**
REF **DP020K ➔ 6 vials of 1 mL.**

Factor VII Deficient plasma
REF **DP030A ➔ 1 vial of 1 mL.**
REF **DP030K ➔ 6 vials of 1 mL.**

Factor X Deficient Plasma
REF **DP060A ➔ 1 vial of 1 mL.**
REF **DP060K ➔ 6 vials of 1 mL.**

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of human origin. Whenever human plasma is required for the preparation of these reagents, approved methods are used to test the plasma for the antibodies to HIV 1, HIV 2 and HCV, and for hepatitis B surface antigen, and results are found to be negative. However, no test method can offer complete assurance that infectious agents are absent. Therefore, 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* diagnostic 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.

DP 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.

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

- **24 hours** at 2-8°C.
- **8 hours** at room temperature (18-25°C).
- **2 months** 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/AR021K/AR021L/AR021M/AR021N).
- Calcium Thromboplastin.
- Specific calibrators and controls with known titration, such as:

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

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

Materials:

- Electromagnetic water-bath, semi-automatic or automatic instrument 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[4] guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references[4,5,6].

PROCEDURE:

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 Factor to measure.** Using this 1:10 diluted preparation, the calibration curve is obtained as follows:

Dilution	1:160	1:80	1:40	1:20	1:10
Factor II, X, VII or X (%)	**6.25***	**12.5**	**25**	**50**	**100**
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

**this complementary dilution should be used when high accuracy is required for the low range (≤10%).*

The calibration curve can also be established with the BIOPHEN™ Plasma Calibrator (222101), using the Factor to measure activity (C) indicated on the flyer for the lot used. The calibration curve must be prepared just before running the assay.
The calibration curve must be used within 2 hours at room temperature (18-25°C).

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

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

3. Dispense the following to the test tube or cuvette:

	Volume
Deficient plasma	100 µL
Calibration point, or tested plasma or controls diluted **1:10**	100 µL
Mix and incubate **at 37°C for 1 minute,** then add the following (starting the stopwatch):	
Calcium Thromboplastin preincubated at **37°C**.	200 µL
Record the exact clotting time (CT) (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 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 method, plot the calibration curve log-log, with the clotting time (sec) along the Y-axis and the factor to measure concentration, expressed as %, along the X-axis.
- The concentration of factor to measure (%) in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.
- 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.
- 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.
- 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 will be divided by 2), and/or another sample and/or another method; check potential associated factor(s) deficiency.
- Thrombin inhibitors present in the tested sample may lead to an underestimation of the Factor to measure concentration.

EXPECTED VALUES:

The normal factor VII level for adult plasma is usually > 60% and the normal factor II, V and X levels for adult plasma is usually > 70%[7]. However, each laboratory has to determine its own normal range.

REFERENCES:

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3. Peyvandi F. *et al.* Rare bleeding disorders. Haemophilia. 2008.
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.
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6. Mauge L. and Alhenc-Gelas M. Stabilité pré-analytique des paramètres de la coagulation: revue des données disponibles. Ann Biol Clin. 2014.
7. Monagle P. *et al.* Impact for clinical haemostasis laboratories. Developmental haemostasis. 2006.

SYMBOLS:

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

Changes compared to the previous version.