Deficient Plasma for Factors VIII:C, IX, XI, XII

CE
IVD

REF DP040A / K DP 1 x 1 mL / 6 x 1 mL
REF DP050A / K DP 1 x 1 mL / 6 x 1 mL
REF DP070A / K DP 1 x 1 mL / 6 x 1 mL
REF DP080A / K DP 1 x 1 mL / 6 x 1 mL

Deficient plasma for Factors VIII:C, IX, XI and XII assay by clotting assay.

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INTENDED USE:

The Factor VIII :C Deficient Plasma, Factor IX Deficient Plasma, Factor XI Deficient plasma and Factor XII Deficient Plasma kits are respectively proposed for the quantitative determination of Factor VIII:C (FVIII:C), Factor IX (FIX), Factor XI (FXI) or Factor XII (FXII) activity in human citrated plasma using a clotting method, in the presence of cephalin, activator (aPTT reagent) and calcium, via a manual or automated method.

SUMMARY AND EXPLANATION:

Technical[1]:

The intrinsic pathway is triggered by contact activation of Factor XII, which results in sequential proteolytic activation of FXI and FIX. Activated FIX then forms with its cofactor (activated FVIII), tenase complex on phospholipid surface to activate Factor X.

Clinical[1,2,3]:

Most affected individuals with FXII deficiency are asymptomatic, but rarely there may be a mild tendency to bleed. The hemorrhagic tendency in individuals with FXI deficiency is often mild, even in patients with homozygous disease, and symptoms do not always relate to the measured level of FXI. The congenital bleeding disorder caused by the deficiency of FVIII or FIX is called haemophilia A and B respectively. Hemophilia may be classified as severe, moderate, or mild, based on the plasma levels of FVIII or FIX in affected individuals. Congenital or acquired deficiencies of intrinsic system clotting factors exhibit a prolonged activated partial thromboplastin time (aPTT).

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 clotting is triggered with cephalin, activator (aPTT reagent) and calcium. Factor to measure is the limiting factor and the clotting time is inversely proportional to the concentration of factor to measure. There is an inverse linear relationship, on a bilogarithmic graph paper, between the factor to measure concentration and the corresponding clotting time.

REAGENTS:

DP **Citrated human plasma, deficient for factor to measure (FVIII:C, FIX, FXI or FXII)**, lyophilized in the presence of glycine and stabilizers. This plasma is deficient for factors to measure (FVIII :C, FIX, FXI or FXII) (<1%), whereas all the other coagulation Factors are within about the normal range (> 50%).

Factor VIII :C Deficient Plasma
REF **DP040A ➔ 1 vial of 1 mL.**
REF **DP040K ➔ 6 vials of 1 mL.**

Factor IX Deficient Plasma
REF **DP050A ➔ 1 vial of 1 mL.**
REF **DP050K ➔ 6 vials of 1 mL.**

Factor XI Deficient plasma
REF **DP070A ➔ 1 vial of 1 mL.**
REF **DP070K ➔ 6 vials of 1 mL.**

Factor XII Deficient Plasma
REF **DP080A ➔ 1 vial of 1 mL.**
REF **DP080K ➔ 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 30 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).
- $CaCl_2$ 0.025 M (AR001B/AR001K/AR001L).
- aPTT reagent (CEPHEN™, CK511K, CK512K, CK515K, CK515L).
- 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 VIII:C, IX, XI or XII) (%)	**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:	
aPTT reagent (cephalin)	100 µL
Mix and incubate **at 37°C for exactly 3 minutes,** then add the following (starting the stopwatch) :	
Calcium Chloride 0.025M preincubated at **37°C**.	100 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 VIII:C level for adult plasma is usually in the range of 50 to 150% and the normal factor IX, XI and XII levels for adult plasma is usually > 60%[7]. However, each laboratory has to determine its own normal range.

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SYMBOLS:

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

Changes compared to the previous version.