FIX Deficient Plasma

REF DP050A / DP050K

R1 1 x 1 mL (DP050A)

R1 6 x 1 mL (DP050K)

Deficient plasma for the assay of FIX with a clotting assay

Not for Sale in the US

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INTENDED USE:

The kit is proposed for the determination of Factor IX (FIX) activity in human citrated plasma using a clotting method, in the presence of cephalin, activator and calcium (aPTT reagent).

PRINCIPLE:

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

REAGENTS:

R1 **Citrated human plasma, deficient for FIX**, immuno-depleted, lyophilized in the presence of glycine and stabilizers. This plasma is deficient for FIX (<1%), whereas all the other coagulation factors are within about the normal range (> 50%).

1 vials of 1 mL (DP050A).
6 vials of 1 mL (DP050K).

WARNINGS AND PRECAUTIONS:

- Biological products must be handled with all necessary precautions and considered as being potentially infectious.
- Waste should be disposed of in accordance with applicable local regulations.
- Handle the reagents with care to avoid contamination during use. If possible, avoid reagent evaporation during use by limiting the liquid-air exchange surface. Evaporation reduces the reagent's stability in the analyzer.
- To preserve reagent stability, seal the vials after use with their respective caps.
- Aging studies, conducted over a 3-week period at 30°C, show that the reagents can be shipped at room temperature over a short period of time, without degradation.
- The human plasma used has been tested by recorded methods and is certified free of HIV antibodies, Hbs Antigen and HCV antibodies.
- For *in vitro* diagnostic use.

REAGENT PREPARATION AND STABILITY:

The reagents are lyophilized under a vacuum in their vials. To avoid any product loss when opening the vial of lyophilized reagents, gently remove the freeze-drying stopper.

R1 **Reagent 1**: **Citrated human plasma, deficient for FIX**

Reconstitute the contents of each vial with exactly **1 mL distilled water**, shake vigorously until fully dissolved. Allow to stabilize for 30 min. at room temperature (18-25 °C), shaking occasionally.
Homogenize the reagent prior to use.
Reagent stability after reconstitution, excluding any contamination or evaporation, and stored in the original vial, is of:

- **24 hours** at 2-8°C.
- **8 hours** at room temperature (18-25°C).
- **2 months** frozen at -20°C or less*

*Thaw only once, as rapidly as possible at 37°C, adapting the incubation period to the volume of reagent**.** The stability of the thawed reagent should be checked under laboratory work conditions.

STORAGE CONDITIONS:

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.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- Imidazole buffer (AR021A/AR021K/AR021L).
- $CaCl_2$ 0.025 M (AR001A/AR001K).
- aPTT reagent (Cephalin, 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

Materials:

- Water-bath, semi-automatic or automatic instrument for clotting assays
- Stopwatch.
- Pipettes with dispensing volumes of 20 µL, 50 µL and 100 µL; or with a variable dispensing volume from 50 µL to 1,000 µL.

SPECIMEN COLLECTION AND PREPARATION:

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

- Specimens:

Human plasma obtained from anticoagulated blood (trisodium citrate).

- Collection:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M) by clean venipuncture. Discard the first tube. Add other anticoagulants depending on the reagent and/or analyte (ex: heparin assay)

- Centrifugation:

Within 2 hours, use a laboratory-validated method to obtain platelet-poor plasma, for example at least 15 minutes at 2500 g at room temperature (18-25°C) and allow the plasma to settle in a plastic tube.

- Plasma storage:
  - 4 hours at room temperature (18-25°C).
  - 1 month at -20°C.
  - 18 months at -70°C[2].

Frozen plasma specimens should be thawed rapidly at 37°C, then shaken thoroughly and tested immediately. Resuspend any precipitate by shaking vigorously immediately after thawing and before use.

PROCEDURE:

Automated methods:

Applications for the various analyzers are available on request. **See the specific application and specific precautions for each analyzer.**

Assay method:

1. Reconstitute the calibrators and controls as indicated in the specific instructions. Prepare 1 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 FIX.** Using this preparation, the calibration curve is obtained as follows:

Dilution	1:160	1:80	1:40	1:20	1:10
FIX (%)	6.25*	12.5	25	50	100
Plasma pool 1:10 or calibrator	0.060mL	0.125mL	0.250mL	0.500mL	1mL
Volume Imidazole Buffer	0.900mL	0.875mL	0.750mL	0.500mL	0mL

**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 IX activity (C) indicated on the flyer for the lot used.

The calibration curve must be used within 1 hour at room temperature (18-25°C).

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

3. In a test tube, or a cuvette, introduce:
- 100 µL of FIX deficient Plasma.
- 100 µL of calibration solution or of tested plasma diluted **1:10**.

Incubate for 1 min. at 37°C, and then introduce:
- 100 µL of aPTT reagent (cephalin)

Incubate for exactly 3 min. at 37°C, and then introduce (starting the stop watch):
- 100 µL of Calcium Chloride 0.025M preincubated at 37°C.

Record the clotting time.

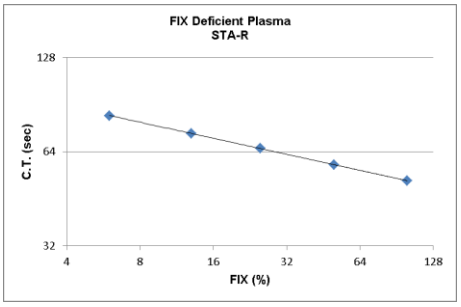
Establish the calibration curve and test it with the quality controls. If stored at room temperature (18-25°C), test the diluted specimens within 2 hours. The exact calibrator and control concentrations for each batch are indicated on the flyer provided with the kit.

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

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.

The calibration curve shown below, obtained with CEPHEN (CK511K, CK512K, CK515K, CK515L) and $CaCl_2$ at 0,025 M (AR001A/AR001K) on STA-R®, is given by way of example only. The calibration curve established for the assay series must be used.



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 defined, 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 acceptable range for the method.
Each laboratory must define its acceptable 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 FIX concentration, expressed as %, along the X-axis.
- The concentration of FIX in the test specimen is directly inferred from the calibration curve, if 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. The laboratory is responsible for validating any changes made to these instructions for use.
- 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.
- Any plasma displaying a coagulum or showing signs of contamination 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 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 FIX concentration.

NORMAL VALUES:

Normal values for Factor IX activity are usually > 60%[3,4,5,6].

APPLICATIONS:

- The reagent is proposed for measuring Factor IX activity, by clotting assay.
- Lyophilized, human citrated plasma, deficient for FIX, for any in vitro protocol or research study where a source of human FIX deficient plasma is required.

ASSAY VARIATIONS:

The obtained clotting times and assay performances can slightly vary according to the cephalin 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, and the normal range, must then be confirmed (and adjusted if necessary) in the laboratory working conditions.

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
2. Woodhams B. *et al*. Stability of coagulation proteins in frozen plasma. Blood coagulation and Fibrinolysis. 2001.
3. **V**an Hylckama Vlieg A, *et al*. "High levels of factor IX increase the risk of venous thrombosis", Blood, 95(12):3678-82, 2000.
4. Taran LD, "Factor IX of the blood coagulation system: a review", Biochemistry (Mosc.), 62(7):685-93, 1997.
5. Orstavik KH, *et al*. "Detection of carriers of haemophilia B", Br J Haematol, 42(2):293-301, 1979.
6. Abrégés "Hémorragies et thromboses – Du Diagnostic au traitement", Samama et coll. Masson, 2004

SYMBOLS:

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