

FIX Deficient Plasma

ADP050A-RUO / ADP050K-RUO

Deficient plasma for the assay of FIX with a clotting assay

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.



Manufactured By: HYPHEN BioMed

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Intended use:

The kit is proposed for the measurement of Factor IX (FIX) activity in human citrated plasma using a clotting method, in the presence of cephalin, activator and calcium (APTT reagent). **This kit is for research use only and should not be used for patient diagnosis or treatment.**

Assay principle:

The method is 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 bilogarithmic graph paper, between the FIX concentration and the corresponding clotting time.

Assay specimen:

Human plasma obtained from Trisodium Citrate anticoagulated blood.

Reagents:

1 vial of 1 ml (DP050A) or 6 vials of 1 ml (ADP050K) of 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%).

Reagents and material required, but not supplied:

- 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.
- Semi-automatic or automatic coagulation instrument, or fibrometer or electromagnetic water bath, stop watch.
- Distilled water.
- Calcium chloride 0.025M (ex AAR001A/AAR001K).
- Imidazole buffer (ex AAR021A/AAR021K/AAR021L).
- Normal human citrated plasma pool or FIX calibrator (BIOPHEN Plasma Calibrator - # A222101).
- Normal and Abnormal quality control plasmas, titrated for FIX (BIOPHEN Normal Control Plasma - #A223201 and BIOPHEN Abnormal Control Plasma - #A223301).
- aPTT reagent (cephalin)

Reagent preparation and stability:

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

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:

When opened and protected from any contamination, the reconstituted plasma is stable for:

- 8 hours at room temperature (18-25°C)
- 24 hours at 2-8°C
- 2 months, frozen at -20°C or below, in its original vial, or in a plastic tube (before use, thaw in a water bath at 37°C, for at least 15 min).

Note: Plasmas used for the Deficient Plasma preparation were tested with registered methods and found negative for HIV antibodies, HBs Ag and HVC antibodies. However, no assay may warrant the total absence of infectious agents. Any product of human origin must then be handled with all the required cautions, as being potentially infectious.

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

Sample collection and preparation:

Blood (9 volumes) must be collected on 0.109 M citrate anticoagulant (1 volume), with great care, in a silicon glass or a plastic tube. Sampling must be performed through a net venipuncture, avoiding any blood activation. Within 2 hours, blood must be centrifuged at 2,500 g for 15 min, and plasma decanted into a plastic tube, using a plastic pipette.

Storage of plasma: up to 4 hours at Room Temperature (18-25°C), or up to 1 month frozen at -20°C or below (before use, thaw 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.

Protocol:

Calibration curve:

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:

IX	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

*these complementary dilutions can 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).

Preparation of tested plasma:

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

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

Assay:

Manual Method:

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.

Automatic Method:

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

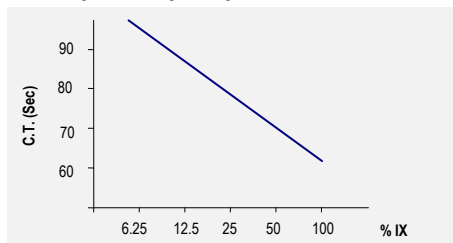
The usual program used for testing the factors involved in the coagulation pathway with a clotting based activated partial thromboplastin time method, and a specific deficient plasma, can be applied. The respective specimen and reagent volume ratios indicated for the manual method must be strictly respected. 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.

Expression of results:

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

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



Quality control:

The control is performed using commercially available control plasmas, titrated for FIX activity. Various control plasmas are available: BIOPHEN Normal Control Plasma: (ref A223201); BIOPHEN Abnormal Control Plasma: (ref A223301). 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.

Cautions and limitations:

- 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 FIX concentration.
- The results obtained should be for research purposes only and not used for patient diagnosis or treatment.

Normal values:

Normal values for Factor IX activity are usually > 60%.

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 clotting times observed for this assay are obtained with CK Prest® from Diagnostica Stago. They are expected <80 seconds for the 100% FIX concentration. 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:

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4. www.ncbi.nlm.nih.gov, OMIM, Haemophilia B, FIX deficiency, +306900, +134540, +134510, +134520.
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