HYPHEN BioMed
www.hyphen-biomed.com
155, rue d'Eragny
95000 NEUVILLE SUR OISE
FRANCE
Tel. : +33 (0)1 34 40 65 10
Fax : +33 (0)1 34 48 72 36
info@hyphen-biomed.com

Factor XII Deficient Plasma

DP080A-RUO / #DP080K-RUO

Deficient plasma for the assay of Factor XII with a clotting assay

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

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Intended use:
The kit is proposed for the measurement of Factor XII (FXII or factor Hageman) 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 FXII, which is brought by the diluted tested plasma, and clotting is triggered with cephalin, activator and calcium (APTT). FXII is the limiting factor and clotting time is inversely proportional to the concentration of FXII. There is an inverse linear relationship, on a bilogarythmic graph paper, between the FXII concentration and the corresponding clotting time.

Assay specimen:
Human plasma obtained from Trisodium Citrate anticoagulated blood.

Reagents:
1 vial of 1 ml (#DP080A-RUO) or 6 vials of 1 ml (#DP080K-RUO) of citrated human plasma, deficient for Factor XII, immuno-depleted, lyophilized in the presence of glycine and stabilizers.
This plasma is deficient for FXII (<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 AR001A/AR001K)
- Imidazole buffer (ex AR021A/AR021K/AR021L).
- Normal human citrated plasma pool or Factor XII calibrator.
- Normal and Abnormal quality control plasmas, titrated for Factor XII.
- 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 vol.) must be collected on 0.109M trisodium citrate anticoagulant (1 vol.); plasma supernatant is decanted following a 20 min. centrifugation at 2,500 g; citrated plasma must be tested within 4 hours when stored at room temperature (20-25°C).

Refer to GEHT or NCCLS/CLSI guidelines for further instructions on specimen collection, handling and storage. Discard any sample with an unusual aspect.

Protocol:
- **Calibration curve:**

Prepare 2 ml of normal citrated human pooled plasma **diluted 1:10** in Imidazole buffer (eg 200µl of pool + 1800µl of buffer). By definition, this ten fold dilution of the normal citrated human plasma pool corresponds to a concentration of **100% of FXII.** Using this preparation, the calibration curve is obtained as follows:

XII	12.5%	25%	50%	100%
Dilution*	**1:80**	**1:40**	**1:20**	**1:10**
Plasma pool 1:10	0.125 mL	0.250 mL	0.500 mL	1 mL
Imidazole Buffer	0.875 mL	0.750 mL	0.500 mL	0 mL

**Complementary dilutions (2 step serial dilutions from the 1:80) could be used when high accuracy is required for the low range (≤10%).*

The calibration curve must be used within 2 hours 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 2 hours.

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 FXII 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):

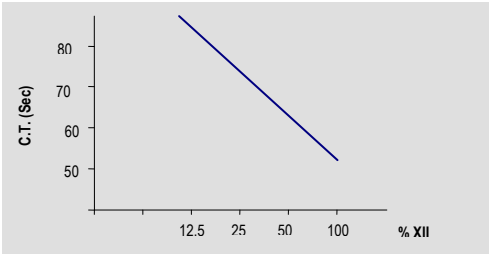
- 100 µl of Calcium Chloride 0.025M preincubated at 37°C.

Record the clotting time.

Automatic Method:
The assay can be used with the semi-automatic or automatic instruments, such as STA-R, KC-4, 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 bilogarythmic graph paper, plot on abscissae the FXII concentrations and on ordinates the corresponding clotting times. On the calibration curve obtained, interpolate directly the corresponding FXII concentration for the tested plasma.

Example of Calibration curve: This calibration curve is indicated as an example only. It was obtained with CEPHEN reagent from HYPHEN BioMed, using the STAR instrument.



QUALITY CONTROL:
The control is performed using commercially available control plasmas, titrated for FXII activity.
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 test plasma; check potential associated factor(s) deficiency.
- Thrombin inhibitors present in the tested sample may lead to an underestimation of the FXII concentration
- **The results obtained should be for research purposes only and not used for patient diagnosis or treatment.**

Normal values:
Normal values for Factor XII activity are usually > 60%.

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

Assay variations:
The clotting times observed for this assay are obtained with eg with CEPHEN reagent from HYPHEN BioMed. They are expected <65 seconds for the 100% FXII concentration. The obtained clotting times and assay performances can vary according to the cephalin reagent type and lot, and the instrument and protocol 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.