BIOPHEN™ Factor XIII

REF 227005

R1 3 x 4 mL, R2 3 x 5 mL

IVD

Chromogenic method for the determination of Factor XIII.

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INTENDED USE:

BIOPHEN™ Factor XIII kit is a chromogenic method for *in vitro* quantitative determination of Factor XIII activity in human citrated plasma.

SUMMARY AND EXPLANATION:

Technical:

Factor XIII (FXIII) protransglutaminase circulates in plasma as A_2B_2 tetramer, at a plasmatic concentration from 14 to 28 mg/L[2], the A subunit being the functional form. When activated by thrombin and calcium to FXIIIa, it acts in the last step of the coagulation cascade and contributes to Fibrin crosslinking and clot stiffness.

Clinical:

FXIII deficiency may be congenital, or acquired as a result of hyperconsumption or presence of autoantibodies. Low FXIII levels have been associated with bleeding complications, eg in situations such as trauma or surgery. FXIII is also involved in various other processes such as wound healing and maintenance of pregnancy.[1,2,3]
Assay of FXIII activity in human plasma may help in the diagnosis of congenital or acquired FXIII deficiency.

PRINCIPLE:

Factor XIII (FXIII), in the tested sample, is converted into activated Factor XIII (FXIIIa) by thrombin in presence of calcium[4]. Soluble fibrin, also generated by the action of thrombin, accelerates the reaction while an anti-polymerization peptide avoids the formation of the clot. FXIIIa transglutaminase activity between a synthetic peptide substrate and glycine ethyl ester (GEE) leads to the formation of ammonia ($NH4^+$). Ammonia is then assayed through the reaction of glutamate dehydrogenase (GLDH) converting NADPH into $NADP^+$, in the presence of ammonia and alpha ketoglutarate. The conversion of NADPH into $NADP^+$ can be detected at 340 nm, and the slope of the absorbance decrease at 340nm is directly proportional to the concentration of FXIII in the tested sample.

$$\text{FXIII} \xrightarrow[\text{Fibrin}]{\text{Thrombin, } Ca^{++}} \text{FXIIIa}$$

$$\text{Glycine Ethyl Ester + peptide} \xrightarrow{\text{FXIIIa}} \text{[Peptide-GEE]} + NH4^+$$

$$NH4^+ + \text{NADPH} + \alpha\text{ ketoglutarate} \xrightarrow{\text{GLDH}} NADP^+ + \text{Glutamate} + H_2O$$

REAGENTS:

R1 **Thrombin reagent**, lyophilized. Contains BSA.
R2 **Detection reagent**, lyophilized. Contains BSA and GEE.

R1 **3 vials of 4 mL.**
R2 **3 vials of 5 mL.**

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of animal origin. 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.

R1 Reconstitute the contents of each vial with exactly **4 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.

R2 Reconstitute the contents of each vial with exactly **5 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.

R1 R2 Reagent stability after reconstitution, free from any contamination or evaporation, and stored closed, is of:

- **1 week** at 2-8°C.
- **48 hours** at room temperature (18-25°C).
- **6 months** frozen 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.
- Physiological Saline (0.9% NaCl).
- Specific calibrators and controls with known FXIII titration, traceable to the International Standard for FXIII in plasma, such as:

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

Materials:

- Spectrophotometer or automatic analyzer for chromogenic assays **at 340nm.**
- Stopwatch; Calibrated pipettes.

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[5] guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references[5].

PROCEDURE:

The kit can be used for kinetics automated method. Perform the test at **37°C** and read color intensity at **340nm**.

Assay method:

1. Reconstitute the calibrators and controls as indicated in the specific instructions. For preparing the calibration curve, dilute the calibrator in physiological saline to calibrate from approximately 0 to 150% FXIII. The **1:2 working dilution in physiological saline (in the schema below)** corresponds by definition to 100% for a normal plasma pool, or C% FXIII for a commercial calibrator.

Calibrator % FXIII	C	C:2	C:4	C:8	0
Volume calibrator	500µL	250µL	125µL	60µL	0µL
Volume Physiological Saline	0µL	250µL	375µL	420µL	500µL

The point **3C/2** (or 150% for a normal plasma pool) is obtained by addition of 30 µL calibrator + 10 µL physiological saline in the table below.

2. Establish the calibration curve and test it with the quality controls.
The exact calibrator and control concentrations for each batch are indicated on the flyer provided with the kit.

3. As an example, the here below table shows the schema for application on CS-series. Dispense the following to the reaction cuvettes incubated at **37°C** (directly managed by the analyzer):

	Volume
Specimen, calibrator or control	20 µL
Physiological saline	20 µL
R1 **Thrombin reagent**, pre-incubated at **37°C**	80 µL
Mix and incubate **at 37°C for exactly 110 seconds**, then add the following:	
R2 **Detection reagent**, pre-incubated at **37°C**	100 µL
Mix, incubate at 37°C, and measure (kinetics mode) the optical density (OD)/min at **340 nm** between **200 and 500 seconds**	

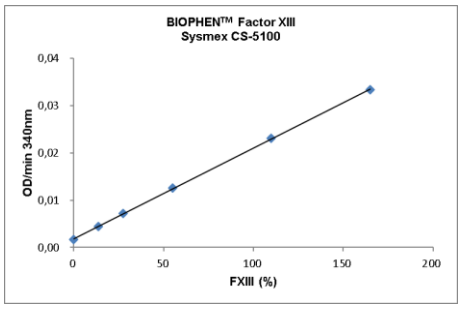
If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

For an automated method, application guides are available on request. See specific application guide and specific precautions for each analyzer.

CALIBRATION:

The BIOPHEN™ Factor XIII assay can be calibrated for the assay of FXIII activity in plasma.

The calibration curve shown below, obtained on Sysmex CS-5100 analyzer, is given as an 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 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:

- On Sysmex CS-series analyzer, the calibration curve lin-lin is obtained, with the OD 340 nm along the Y-axis and the FXIII concentration, expressed as %, along the X-axis.
- The concentration of FXIII (%) 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.

EXPECTED VALUES:

The reference range established on healthy adult subjects (n=120) using Sysmex CS-5100 (Central 90%, 95th percentile) was measured between 60 and 146%.
However, each laboratory has to determine its own normal range.

PERFORMANCES:

- The lower analyzer detection limit depends on the analytical system used (<0.5% on Sysmex CS-5100).
- The measuring range depends on the analytical system used (about 5 to 300% of FXIII on Sysmex CS-series, the test being linear from 5 to 150% without redilution).
- Performance studies were conducted internally on Sysmex CS-5100. Performance was assessed using laboratory controls over a 5-day period, 2 series per day and 3 repetitions within each series for a control level. The following results were obtained:

Control	Intra assay				Inter assays			
	n	Mean	CV%	SD	n	Mean	CV%	SD
Normal	40	102.3	2.7	2.8	30	102.6	1.5	1.5
Abnormal	40	28.8	4.9	1.4	30	31.2	1.9	0.6

- Correlation with reference method (Berichrom FXIII (Siemens) vs BIOPHEN™ Factor XIII (HBM) on Sysmex CS-2500) :
  n = 102 y = 0.90x – 5.84 r = 0.989
- Interferences:
  No interference, on the analyzer Sysmex S-5100 was observed with the molecules and up to following concentrations:

Hemoglobin	Bilirubin (F/C)	Intralipids	Fibrinogen	Heparins (UFH/LMWH)
250 mg/dL	60 mg/dL	250 mg/dL	0.8 – 6 g/L	2 IU/mL
Ammonium	**Dabigatran**	**Rivaroxaban**	**Apixaban**	**Edoxaban**
0.5 mM	400 ng/mL	400 ng/mL	400 ng/mL	400 ng/mL

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

REFERENCES:

1. Menegatti M. *et al*., Minimal factor XIII activity level to prevent major spontaneous bleeds. J Thromb Haemost. 2017.
2. Komaromi I. *et al*., Factor XIII, novel structural and functional aspects. J Thromb Haemost .2011.
3. Schroeder V. and Kohler HP. New developments in the area of factor XIII. J Thromb Haemost. 2013.
4. Karpati L. *et al.* A modified, optimized kinetic photometric assay for the determination of blood coagulation factor XIII activity in plasma. Clin Chem. 2000.
5. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008.

SYMBOLS:

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

R2 H318: Causes serious eye damage.

Changes compared to the previous version.