BIOPHEN™ Factor XIII

REF 227005

R1 3 x 4 mL, R2 3 x 5 mL

IVD

Chromogenic method for the determination of Factor XIII.

HYPHEN BioMed

Sales and Support:
CoaChrom Diagnostica GmbH
www.coachrom.com | info@coachrom.com
Tel: +43-1-236 222 1 | Fax: +43-1-236 222 111
Toll-free contact for Germany:
Tel: 0800-24 66 33-0 | Fax: 0800-24 66 33-3

English, last revision: 11-2017

INTENDED USE:

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

SUMMARY AND EXPLANATION:

Factor XIII (FXIII) protransglutaminase circulates in plasma as A_2B_2 tetramer, 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. 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.
3 vials of 4 mL.
R2 **Detection reagent**, lyophilized. Contains BSA and GEE.
3 vials of 5 mL.

WARNINGS AND PRECAUTIONS:

- Biological products must be handled with all necessary precautions and considered as being potentially infectious.
- This material contains substances of animal origin and must be handled as a carrier and a potential transmitter of diseases.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.
- 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.
- For *in vitro* diagnostic use.

R2 GEE: H318: Causes serious eye damage.

REAGENT PREPARATION AND STABILITY:

The reagents are lyophilized under 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: Thrombin reagent**

Brown vial. Reconstitute the contents of each vial with exactly **4 mL distilled water**, shake vigorously until fully dissolved, while avoiding formation of foam. 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:

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

R2 **Reagent 2: Detection reagent**

White vial. Reconstitute the contents of each vial with exactly **5 mL distilled water**, shake vigorously until fully dissolved, while avoiding formation of foam. 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:

- **1 week** at 2-8°C.
- **48 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.
- Physiological Saline (0.9% NaCl).
- Specific calibrators and controls with known FXIII titration, traceable to the International Standard for FXIII in plasma.

Materials:

- Automatic instrument for chromogenic assays with wavelength at **340 nm**.
- Calibrated pipettes; Plastic tubes.

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[5] guidelines for further information concerning specimen collection, handling and storage).

- 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.

- Centrifugation:

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

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

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:

The kit can be used in kinetics mode on automated methods. Perform the test at **37°C** and read the absorbance at **340nm**.

Automated methods:

See the specific application and specific precautions for each analyzer (provided on request for various instruments according to availability; contact your local distributor for CS-series applications).

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**	

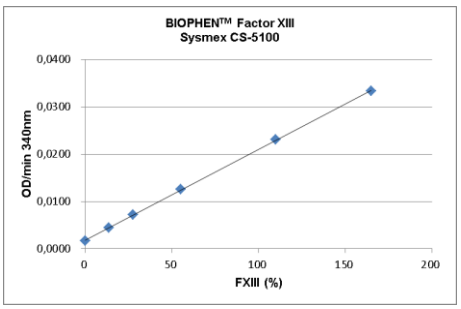
If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

CALIBRATION:

The BIOPHEN™ Factor XIII assay can be calibrated for the assay of FXIII activity in plasma.
Using a linear scale:

- The test is linear from 5 to 150% of FXIII on Sysmex CS-5100 (at the standard dilution).

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-run reproducibility for a given lot of reagents.
Include 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 lot, or after analyzer maintenance, or when the measured quality control values fall outside the acceptable range.
Each laboratory must define its acceptable ranges and verify the expected performance in its analytical system.

RESULTS:

- On the Sysmex CS-series analyzer, the calibration curve is obtained in Lin-Lin scale, with the OD/min at 340 nm along the Y-axis and the FXIII concentration, expressed as %, along the X-axis.
- The concentration of Factor XIII in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.
- Results are expressed in percentage.
- 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 plasma displaying a coagulum or showing signs of contamination must be rejected.
- For the possible influence of interferences, refer to specific application for the analyzer used (no significant effect is observed on Sysmex CS-5100 for Heparin concentration up to 2 IU/mL, bilirubin concentration up to 60 mg/dL, hemoglobin concentration up to 250 mg/dL, intralipids concentration up to 250 mg/dL, ammonium concentrations up to 0.5mM, and fibrinogen concentrations from 0.8 up to 6 g/L, by plasma overload tests. For high concentrations, an additional (eg 1:3) pre-dilution could be used and the result multiplied by the complementary dilution factor).

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).
- On Sysmex CS-series, the measuring range is from about 5 to 300% of FXIII.
- Performance studies were conducted internally on 1 batch of reagent using a Sysmex CS-5100. Performance was assessed using laboratory controls over a 5-day period, 2 series per day and triplicates 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

REFERENCES:

1. Menegatti et *al*., Minimal factor XIII activity level to prevent major spontaneous bleeds. J Thromb Haemost, 15:1728-1736, 2017.
2. Komaromi et *al*., Factor XIII, novel structural and functional aspects. J Thromb Haemost 2011; 9:9-20.
3. Schroeder V, Kohler HP. New developments in the area of factor XIII. J Thromb Haemost 2013; 11: 234–44.
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.