Berichrom® F XIII

Revision bar indicates update to previous version.

CE 0197

Intended Use

Berichrom® F XIII is an in vitro diagnostic reagent for the quantitative, WHO-standardized determination of FXIII activity as aid to diagnosis and monitoring of congenital or acquired FXIII deficiencies in patients at risk for or suspected to have FXIII deficiency in human sodium citrated plasma by means of automated, chromogenic methods.

In addition, Berichrom® F XIII assay can be used for monitoring FXIII substitution therapy.

Summary and Explanation

Coagulation factor XIII (FXIII) is found in plasma and platelets. Plasma FXIII consists of 2 A-subunits and 2 B-subunits. After FXIII is activated by thrombin, the transglutaminase FXIIIa catalyzes the formation of peptide bonds between adjacent molecules of fibrin monomers, thus providing mechanical and chemical stability to the fibrin clot. Fibrin that is not covalently cross-linked exhibits an increased susceptibility to fibrinolysis[1,2].

Congenital FXIII deficiency is an autosomal recessive bleeding disorder. Affected individuals experience soft tissue hemorrhage, hemarthrosis, and hematomas. Homozygous FXIII deficiency ls very rare with an estimated prevalence of 1 in 4 million, usually exhibiting FXIII levels < 10 %. Severe inherited FXIII deficiency with levels <4 – 5 %are associated with a high risk of intracranial hemorrhage and most affected individuals are on long-term prophylaxis with FXIII concentrate[1–5]. Heterozygous carriers may be asymptomatic; however, females may experience recurrent spontaneous abortions[1,2]. Acquired FXIII deficiency can develop due to increased consumption of FXIII or due to the presence of FXIII specific inhibitors[6].

Determination of FXIII activity is the preferred method for screening and diagnosis of FXIII deficiency[3,5,7,8].

The determination of FXIII activity in plasma is indicated in the following cases:

- diagnosing congenital or acquired factor FXIII deficiency states,
- differential diagnosis in bleeding diathesis
- peri-operative bleeding management
- monitoring FXIII substitution therapy in FXIII deficiency

Principles of the Procedure

FXIII contained in the sample is converted by the action of thrombin into FXIIIa. Fibrin formed by thrombin also accelerates this reaction. Fibrinogen is not removed prior to testing since this would cause a loss of FXIII. Instead, fibrin produced by the action of thrombin is prevented from forming clots by an aggregation inhibiting peptide and held in solution.

FXIIIa cross-links a specific peptide substrate to glycine ethyl ester, thereby releasing ammonia. The ammonia released is then determined in a parallel enzymatic reaction. The decrease in NADH is measured by monitoring the absorbance at 340 nm[9].

$$\text{fibrinogen} \xrightarrow{\text{thrombin}} \text{fibrin}_{\text{soluble}} \xrightarrow{\text{clot inhibitor}} \text{clot}$$

$$FXIII \xrightarrow[fibrin]{thrombin} FXIIIa$$

$$\text{glycine ethyl ester + peptide substrate} \xrightarrow{FXIIIa} \text{conjugate + } NH_3$$

$$NH_3 + NADH + \alpha\text{-ketoglutarate} \xrightarrow{GLDH} NAD + \text{glutamate}$$

Reagents

Note: Berichrom® F XIII can be used on automated coagulation analyzers. Sysmex provides Reference Guides (Application Sheets) for several coagulation analyzers. The Reference Guides (Application Sheets) contain analyzer/assay specific handling and performance information which may differ from that provided in these Instructions for Use. In this case, the information contained in the Reference Guides (Application Sheets) supersedes the information in these Instructions for Use. Please also consult the instruction manual of the instrument manufacturer!

Reagent	Description	Storage	Stability
Berichrom® F XIII			

ACTIVATOR	Lyophilized reagent containing:
	• Thrombin, bovine (reconstituted: 10 IU/mL)
	• gly-pro-arg-pro-ala-amide (reconstituted: 2 g/L)
	• Calcium chloride (reconstituted: 1.2 g/L)
	• Hexadimethrine bromide (reconstituted: 10 mg/L)
	• BSA
	• Buffer:
	  • Bicine (reconstituted: 0.1 mol/L)
	• Preservative:
	  • Sodium azide (reconstituted: ≤0.5 g/L)
	pH 8.3
		2–8 °C
		May be used up to the expiry date indicated on the label if stored unopened.
			37 °C: reconstituted, 8 hours;
			15–25 °C: reconstituted, 2 days;
			2–8 °C: reconstituted, 1 week;
			–20 °C: reconstituted, 6 months

Reagent	Description	Storage	Stability
ACTIVATOR DILUENT	Lyophilized reagent containing: • NADH (reconstituted: 0.5 g/L) • BSA • Preservative: • Sodium azide (reconstituted: ≤0.5 g/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	n/a

DETECTION REAGENT	Lyophilized reagent containing:
	• GLDH (reconstituted: 20 IU/mL)
	• synthetic peptide[a] (reconstituted: 2.4 g/L)
	• ADP
	• Glycin-ethylester-hydrochlorid (reconstituted: < 4.0 g/L)
	• α-ketoglutarate (reconstituted: 2.7 g/L)
	• BSA
	• Buffer:
	  • HEPES (reconstituted: 10 mmol/L)
	• Preservative:
	  • Sodium azide (reconstituted: < 1.0 g/L)
	pH 6.5
		2–8 °C
		May be used up to the expiry date indicated on the label if stored unopened.
			37 °C: reconstituted, 8 hours;
			15–25 °C: reconstituted, 2 days;
			2–8 °C: reconstituted, 1 week;
			–20 °C: reconstituted, 6 months

[a] as FXIII substrate

Signs of expiry: Absence of vacuum when opening the vial; reagent is difficult to reconstitute; results are not reproducible.

On-board stability
Information regarding on-board stability is specified in the Reference Guides (Application Sheets) for the different coagulation analyzers.

Stability of the Reagent Mixture

Temperature	Reagent Mixture
37 °C	4 hours
15–25 °C	8 hours
2–8 °C	2 days
–20 °C	2 months

The stability data for the reagent mixture apply if freshly reconstituted individual reagents are used. If the reagent mixture is made from individual reagents that have already been reconstituted and then stored, it must be used immediately.

Warnings and Precautions

For *in-vitro* diagnostic use only.

For laboratory professional use.

According to EU regulation 2017/746, any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State through your local distribution representative in which the user and/or patient is established.

Safety data sheets (MSDS/SDS) available upon request.

Danger! Berichrom F XIII DETECTION REAGENT
Hazardous ingredient: Sodium azide (4.07 % [w/w]), Glycin-ethylester-hydrochlorid (>15.6 % [w/w]).
H311: Toxic in contact with skin. **H302**: Harmful if swallowed. **H318**: Causes serious eye damage. **H411**: Toxic to aquatic life with long lasting effects.
P264: Wash hands thoroughly after handling. **P280**: Wear protective gloves/protective clothing/eye protection/face protection. **P273**: Avoid release to the environment. **P312**: Call a POISON CENTER or doctor/physician if you feel unwell. **P361 + P364**: Take off immediately all contaminated clothing and wash it before reuse. **P391**: Collect spillage. **P305 + P351 + P338**: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. **P310**: Immediately call a POISON CENTER or doctor/physician. **P501**: Dispose of contents and container in accordance with all local, regional, and national regulations.

Danger! Berichrom F XIII ACTIVATOR DILUENT
Hazardous ingredient: Sodium azide (8.33 % [w/w]).
H311: Toxic in contact with skin. **H302**: Harmful if swallowed. **H411**: Toxic to aquatic life with long lasting effects.
P264: Wash hands thoroughly after handling. **P280**: Wear protective gloves/protective clothing/eye protection/face protection. **P273**: Avoid release to the environment. **P312**: Call a POISON CENTER or doctor/physician if you feel unwell. **P361 + P364**: Take off immediately all contaminated clothing and wash it before reuse. **P391**: Collect spillage. **P501**: Dispose of contents and container in accordance with all local, regional, and national regulations.

Warning! Berichrom F XIII ACTIVATOR
Hazardous ingredient: Sodium azide (1.98 % [w/w]).
H302 + H312: Harmful if swallowed or in contact with skin. **H412**: Harmful to aquatic life with long lasting effects.
P264: Wash hands thoroughly after handling. **P280**: Wear protective gloves/protective clothing/eye protection/face protection. **P273**: Avoid release to the environment. **P312**: Call a POISON CENTER or doctor/physician if you feel unwell. **P501**: Dispose of contents and container in accordance with all local, regional, and national regulations.

Berichrom F XIII ACTIVATOR
Hazardous ingredient: Thrombin, bovine (0.265 % [w/w]).
May produce an allergic reaction.

Caution
Berichrom F XIII ACTIVATOR**, Berichrom F XIII** ACTIVATOR DILUENT**, Berichrom F XIII** DETECTION REAGENT
This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

Contains sodium azide as a preservative. Sodium azide can react with copper or lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides. Disposal into drain systems must be in compliance with prevailing regulatory requirements.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with all government requirements.

Summary of Safety and Performance (SSP) is available in the European database on medical devices (see Eudamed public website: https://ec.europa.eu/tools/eudamed). In case Eudamed is not available, SSP can be delivered by the manufacturer on request.

Preparing Reagents

Berichrom F XIII ACTIVATOR DILUENT: Dissolve in 5 mL of distilled or de-ionized water.

Berichrom F XIII ACTIVATOR: Reconstitute with 5 mL Berichrom F XIII ACTIVATOR DILUENT.

Berichrom F XIII DETECTION REAGENT: Dissolve in 5 mL of distilled or de-ionized water.

The further preparation depends on the testing method. For automatic processing with a coagulation analyzer, please refer to the information in the respective Reference Guide/ Application Sheet. Manual testing is described in section "Manual Testing", page 5.

Specimen Collection and Handling

Collecting the Specimen

To obtain the plasma, carefully mix 1 part sodium citrate solution (0.11 mol/L (3.2 %)) with 9 parts venous blood, avoiding the formation of foam. Centrifuge immediately at 1500 × g for no less than 15 minutes at room temperature[10].

Storing the Specimen

Stability of the samples:

–20 °C	2 months
2 to 8 °C	24 hours
15 to 25 °C	8 hours

Procedure

Materials Provided

REF	Contents	
OWSU11	Berichrom® F XIII	
	Berichrom® F XIII Activator Reagent Berichrom F XIII ACTIVATOR	3 × → 5 mL
	Berichrom® F XIII NADH Reagent Berichrom F XIII ACTIVATOR DILUENT	3 × → 5 mL
	Berichrom® F XIII Detection Reagent Berichrom F XIII DETECTION REAGENT	3 × → 5 mL

Materials Required but not Provided

Item	Description
REF ORKL17	STANDARD PLASMA, Standard Human Plasma
REF ORKE41	CONTROL N, Control Plasma N
REF OUPZ17	CONTROL P, Control Plasma P
REF B4234-25 REF B4265-37	OV BUFFER, Dade® Owren's Veronal Buffer, or CA SYSTEM BUFFER, Dade® CA System Buffer
–	Isotonic Saline Solution
Coagulation analyzers[b], such as:	• AUTOMATED BLOOD COAGULATION ANALYZER CS-2500 (CS-2500 System) • AUTOMATED BLOOD COAGULATION ANALYZER CS-5100 (CS-5100 System)

[b] Availability of analyzers may vary by country.

Please note that the applications on other analyzers can be validated by the instrument manufacturer in accordance with the requirements of the REGULATION (EU) 2017/746 under their responsibility as long as the intended purpose and performance are not modified.

Manual Testing

For the test, mix the necessary volume of reagent using equal parts of ACTIVATOR and DETECTION REAGENT. For stability data see section "Stability of the Reagent Mixture", page 3.

Cuvette:	10 mm thickness
Wavelength:	340 nm
Testing temperature:	37 °C

Prewarm the reagent mixture and the plastic cuvettes or tubes to 37 °C before testing.

Sample	100 µL
Reagent Mixture	1 000 µL

Mix, then begin measuring at 340 nm. To measure, use either a recording instrument connected to the photometer and evaluate using the speed of the change in absorbance (ΔA/min) after reaching the linear phase (after about 5 minutes) or read the absorbance after exactly 5 and 10 minutes and calculate the change in absorbance per minute.

Internal Quality Control

Normal range: CONTROL N

Pathological range: CONTROL P

Two levels of quality control material (normal and pathological range) have to be measured at start of the test run, with each calibration, upon reagent vial changes and at least every 8 hours during each day of testing. The controls should be treated like the samples. Each laboratory should determine its own quality control range, either by means of the target values and ranges provided by the manufacturer of the controls or by means of control values determined in the laboratory. If the measured control value lies outside of the pre-determined confidence range, then the reagents, the laboratory-internal factor F_L or the reference curve and the coagulation analyzer should be checked. Do not release patient results until the cause of deviation has been identified and corrected.

Results

The speed of the change in absorbance after reaching the linear phase (after about 5 minutes) is proportional to the FXIII activity.

The results can be evaluated either with a factor (F_L) or with a reference curve. To calculate the factor, STANDARD PLASMA is used as a sample in duplicate determination. To calculate the reference curve, STANDARD PLASMA is used in different dilution steps (with isotonic saline solution) in duplicate determination.

Calculation of the factor:

$$F_L = \frac{\text{Target value (\% of Norm)}_{\text{Standard Human Plasma}}}{\Delta\text{A/min}_{\text{Standard Human Plasma}}}$$

Multiply the ΔA/min of the sample by the factor obtained; this indicates FXIII in % of Norm.

$\text{FXIII}_{\text{Sample}}$ (% of Norm) = $F_L \times \Delta\text{A/min}_{\text{Sample}}$

A new laboratory-internal factor F_L or reference curve must be re-calculated each time there is a change in the device used or a change in the lot of Berichrom® F XIII.

Limitations

FXIII content may be underestimated or overestimated[9,11,12] if ammonia or ammonium levels in the samples are high (> 0.5 mmol/L). If necessary, determine the ammonia concentration and measure for Factor XIII again with the samples diluted in isotonic saline solution, then multiply the results obtained by the dilution factor.

Very low (< 0.8 g/L) and very high (>6.0 g/L) fibrinogen concentrations may lead to a false low result for FXIII activity. If the fibrinogen is over 6.0 g/L, the sample must be prediluted 1:2 or 1:3 with isotonic saline solution.

Unspecific reactivity of sample components can lead to overestimation of FXIII activity[11].

The manufacturer has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. Please note that the applications on other analyzers can be validated by the instrument manufacturer in accordance with the requirements of the REGULATION (EU) 2017/746 under their responsibility as long as the intended purpose and performance are not modified. User defined modifications are not supported by the manufacturer as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Application Sheets or these Instructions for Use.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

Expected Values

70 to 140 % of Norm[13]

Reference intervals vary from laboratory to laboratory depending on the population served and the technique, method, equipment and reagent lot used. Therefore, each laboratory must establish its own reference intervals or verify them whenever one or more of the aforementioned variables are changed.

Performance Characteristics

Measuring Range

The measuring range extends from 5 to 150 % of Norm.

The measuring range depends on the individual application of the assay due to instrument related conditions. Application specific performance data is listed in the respective Reference Guides of the instruments.

Precision

The coefficient of variation within run was 5 % for CONTROL N and 4 % for CONTROL P. The coefficient of variation from day to day was 6 % for CONTROL N and 4 % for CONTROL P.

Other system specific results are given in the respective Reference Guides (Application Sheets).

The reproducibility was assessed by the manufacturer for Coagulation Factor XIII assay with Berichrom® F XIII based on publicly available proficiency testing information in 2020/2021. The overall reproducibility median CV% was found to be <11 % including lot, instrument, laboratory, and operator variability factors.

Method Comparison

For other FXIII determinations (Laurell immunoelectrophoresis, clot stability test), coefficients of correlation of 0.8 and 0.82 were obtained[14].

Technical Assistance

For customer support, contact your local technical support provider or distributor.

Current Version of Application Sheets

Berichrom® F XIII can be used in combination with various automated coagulation analyzers. Sysmex provides Reference Guides/Application Sheets for the coagulation analyzers listed in section "Materials Required but not Provided", page 5 under the dedicated link below:

sysmex-ifu.com/ag

As the manufacturer continuously monitors the product performance and safety, the users are required to ensure that they work with the correct revision of the instructions for the product lots in use. Please periodically review the availability of new electronic labeling revisions to ensure safe use of the product.

The IFU version number is visible on each product box label. Sysmex ensures that all products lots bearing the same IFU version number are compatible with the electronic labeling provided via sysmex-ifu.com.

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Definition of Symbols

The following symbols may appear on the product labeling:

	Do not reuse		Use By
LOT	Batch Code	REF	Catalogue Number
	Caution		Manufacturer
EC REP	Authorized representative in the European Community	CH REP	Authorized representative in Switzerland
	Contains sufficient for <n> tests		Biological Risks
IVD	*In Vitro* Diagnostic Medical Device		Temperature Limitation
	Consult instruction for Use		Non-sterile
CE	CE marking of conformity	CE 0197	CE marking of conformity with notified body ID number. Notified body ID number can vary.
CONTENTS	Contents		Reconstitution volume
LEVEL	Level		Keep away from sunlight and heat
WARNING	Warning	DANGER	Danger
RxOnly	Prescription device (US only)	UDI	Device Identification (UDI) barcode
REACH xx/xx/xx	REACH Authorization Number		

Legal Information

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