REF **5800200 TECHNOFLUOR FXIII Activity** ENG

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TECHNOFLUOR FXIII Activity - English

INTENDED USE

FXIII activity assay based on its isopeptidase function for the automated, quantitative determination of FXIII activity in citrated human plasma, for use in research activities on the Ceveron s100.

SUMMARY

Factor XIII (FXIII) plays a key role in clot stabilization, maturation and composition. The FXIIIA subunit belongs to the transglutaminase family of which the most characteristic catalytic function is the formation of isopeptide bonds between the side chains of susceptible protein bound glutamine and lysine residues. In plasma, the non-covalent FXIII-A_2B_2 heterotetramer is bound to fibrinogen. Activation by thrombin and calcium binding yields activated FXIII (FXIIIa), which recognises fibrin as the key substrate. The activated plasma transglutaminase - in an orchestrated sequence - covalently crosslinks abutting fibrin γ-chains and α-chains thereby providing mechanical stability to the fibrin network. In parallel, FXIIIa incorporates α2-antiplasmin rendering the clot biochemically stable by preventing premature fibrinolysis by plasmin. Congenital or acquired deficiency may be associated with a severe bleeding diathesis, underlining the clinical relevance of FXIII.

In the TECHNOFLUOR assay, FXIII is activated by thrombin in the presence of calcium. Activated FXIII then cleaves the side-chain carboxamide bond of the assay's substrate and thereby releases the dark quencher (2,4-dinitrophenyl) linked to the cadaverine spacer. Subsequently, the increase of fluorescence results from the N-terminally attached fluorophore N-Methyl-2-aminobenzoic acid (N-Me-Abz). The fluorescent signal is proportional to the FXIII activity in the plasma sample.

REAGENTS

The TECHNOFLUOR FXIII Activity contains:

	Reagent / Content	Description
2 x 2 mL	Reagent	Activator reagent containing bovine thrombin, calcium chloride and a fibrin polymerization inhibitor
2 x 2 mL	Substrate (SUB)	Factor XIII specific substrate with dark quencher
2 x 3 mL	Buffer (BUF)	Factor FXIII buffer for sample dilution

Material required (not supplied with the kit)

- Distilled water
- Precision pipettes
- Calibration Plasma*
  REF 5220110 Coagulation Reference 5 x 1 mL
- Control Plasma*
  REF 5020040 Coagulation Control N 5 x 1 mL
  REF 5021055 Coagulation Control A 5 x 1 mL
- Laboratory timer
  * or any other package sizes.

Warning and precautions

- RUO for research use only.
- This kit is intended for use by personnel trained in laboratory procedures and universal precautions for the use of chemicals and potentially biohazardous substances must be applied.
- All human blood or plasma products as well as test samples must be considered as potentially infectious. They have to be handled with appropriate care and in strict observance of safety regulations. The rules pertaining to disposal are the same as applied to disposing hospital waste.
- Get a Material Safety Data Sheet for this product from www.technoclone.com.

Stability and storage

The expiry date printed on the labels is only applicable to storage of the unopened containers at 2…8 °C.

Stability opened/ in use:

Reagent / Content	Ceveron s100 (open vial)	2…8 °C (closed vial)	< -20 °C (closed vial)
Reagent	3 days	1 month	2 months
Substrate (SUB)	3 days	1 month	2 months
Buffer (BUF)	3 days	2 months	Do not freeze

Reagent and Substrate should only be frozen once. Thawing must be performed rapidly in a water bath kept at 37 °C.

TEST PROCEDURE

Preparation of plasma samples

Collect nine parts of freshly drawn venous blood in one part trisodium citrate (3.2 %). Refer to CLSI Document H21-A5 for instructions on specimen collection, handling, and storage.

Thaw frozen samples rapidly at 37 °C and centrifuge and separate if necessary. Gently mix before testing. After thawing, the assay must be performed within 2 hours. Samples may be frozen once at ≤-20 °C.

Preparation of reagents

Before starting the test, all the required components must be brought to room temperature.

Avoid foam formation when reconstituting plasmas and mixing reagents or buffers.

- *Reagent*: Dissolve each bottle of lyophilized reagent in 2.0 mL distilled water and swirl gently. Allow the reconstituted material to stand for 10 minutes at room temperature before use. For standardizing tests, a reconstitution time of 30 minutes is recommended. Swirl to mix before use.
- *Substrate:* Dissolve each bottle of lyophilized substrate in 2.0 mL distilled water and swirl gently. Allow the reconstituted material to stand for 10 minutes at room temperature before use. For standardizing tests, a reconstitution time of 30 minutes is recommended. Swirl to mix before use.
- *Buffer*: The FXIII buffer is ready to use.

Performance of the test

The TECHNOFLUOR FXIII Activity is performed on the Ceveron s100 with the respective application.

Calibration is performed using a serial dilution of Coagulation Reference in assay buffer. Normal and abnormal controls are recommended for a complete quality control program. Coagulation Control N and Coagulation Control A are designed for this program. Each laboratory should establish its own mean and standard deviation for a quality control program in order to monitor laboratory testing. Controls should be analyzed before validating sample results in accordance with good laboratory practice.

LIMITATION OF THE TEST

FXIII results on Ceveron s100 are not affected by hemoglobin up to 500 mg/dL, bilirubin* up to 18 mg/dL, lipemia up to 1400 mg/dL Intralipid™, fibrinogen up to 6 g/L and ammonia up to 2 mM.

*In case of clear visual presence of icterus in the plasma sample, a pre-dilution of the sample 1:2 using 0.9 % sodium chloride solution is recommended. The result from the diluted sample must be multiplied by the dilution factor (x2).

INTERPRETATION OF RESULTS

FXIII Activity results are reported in % of Normality. The results can also be converted into IU/mL where 100 % equals 1.00 IU/mL.

REFERENCE RANGE

Reference range (n= 154) for TECHNOFLUOR FXIII activity: 47 – 136 % (equivalent to 0.47 – 1.36 IU/mL)

It is recommended that individual laboratories establish their own reference range.

PERFORMANCE CHARACTERISTICS

Performance data are given below. Results obtained in individual laboratories may differ.

Clinical Performance

A single center method comparison was performed with samples covering the whole assay range comparing TECHNOFLUOR FXIII Activity with a commercially available ammonia release activity assay and a FXIII antigen assay (n=110).

Method	Slope	Intercept	Correlation
Ammonia Release	1.011	-5.448	0.950
FXIII:Ag	1.124	-1.184	0.980

Precision

Reproducibility was determined with different samples.

Sample code	Assigned value [%]	CV % within run	CV % between run
Coagulation Control N	88.1	4.2 %	6.3 %
Coagulation Control A	36.7	3.1 %	4.1 %
Plasma Sample 1	98.2	3.8 %	7.6 %
Plasma Sample 2	58.3	3.7 %	6.0 %
Plasma Sample 3	29.3	4.8 %	7.5 %
Plasma Sample 4	14.7	4.9 %	9.2 %

Limit of quantification and assay range

Lower Limit of Detection: 0.8 %

Linear Range: 1.0 – 150 %

STANDARDISATION

Coagulation Reference (Calibrator) is calibrated using TECHNOFLUOR FXIII Activity and traceable to the WHO International Standard for FXIII Activity and ranges for Coagulation Control N and Coagulation Control A are assigned via analysis with that calibrator and reagent pairing.

LITERATURE

Please contact Technoclone www.technoclone.com or your local distributor.

EDITORIAL NOTE

This document is available in several languages. The translations have been done using the master document in English. In the event of doubts or discrepancies, the wording in the master document in English shall take precedence.


Manufacturer

LOT Iot


Storage temperature

Σ Determinations


Expiry date /

REF Catalogue number


Consult instructions for use

GTIN Global Trade Item Number


Biological risk

BUF Reaction buffer

RUO Research use only

SUB Substrate

AQUA Distilled water

DIL Dilute or disolve in


Manufacturing date

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