TECHNOCHROM® FXIII
For research use only

GB

REF 5360010 TECHNOCHROM® FXIII 3 x 3 mL

Symbols key			
	Manufacturer		Expiry date
	Storage temperature		Consult instructions for use
AQUA	Distilled water		Determinations
BUF	Reaction buffer	LOT	Lot
CAL	Calibrator	MTP	Microtiter plate
CONJ	Conjugate	REF	Catalogue number
CONT	Control	RTU	Ready to use
DIL	Dilute or dissolve in	STOP	Stop solution
INC	Incubation buffer	SUB	Substrate
RUO	For research use only	WASH	Washing solution concentrate

Technoclone Herstellung von
Diagnostika und Arzneimitteln GmbH
Brunner Str. 67 - 1230 Vienna, Austria

TECHNOCHROM® FXIII

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PRODUCT DESCRIPTION

INTENDEND USE

The TECHNOCHROM® FXIII kit is a reagent kit for the determination of blood coagulation Factor XIII (FXIII) activity to detect inherited or acquired FXIII deficiencies, abnormal FXIII with decreased activity and elevated FXIII level.
Inherited FXIII deficiency is a rare, but severe bleeding diathesis with occasional wound healing impairment and in women with habitual abortion. Acquired FXIII deficiency due to an anti-FXIII autoantibody is also a very severe haemorrhagic diathesis. Consumption of FXIII in various diseases (malignant diseases, Chron disease Henoch-Schoenlein purpura, major surgery, etc…) usually results in moderate decrease of FXIII level. The assay can also be used for monitoring FXIII replacement therapy.
FXIII is transformed into an active transglutaminase active FXIII (FXIIIa) during its activation. The determination of FXIII activity is based on the measurement of ammonia released during the transglutaminase reaction. FXIII present in the plasma sample is activated by thrombin and Ca^{2+}. Fibrin formed from fibrinogen by thrombin accelerates this reaction. The polymerisation of fibrin is prevented by a tetra peptide. The formed FXIIIa then cross-links the amine substrate glycine ethyl ester (GEE) to the glutamine residue of specific peptide substrate PI(1-12), and ammonium ions are released. In the indicator reaction the amount of released ammonia is monitored in a glutamate dehydrogenase (GLDH) catalysed NADPH-dependent reaction. The consumption of NADPH is measured spectrophotometrically by the decrease of absorbance at 340 nm. Within a time window the decrease of absorbance is directly proportional to the FXIII activity.

TEST PRINCIPLE

$$\text{FXIII} \xrightarrow[Ca^{2+}]{\text{Thrombin}} \text{FXIIIa}$$

$$\text{PI(1-12) + GEE} \xrightarrow{\text{FXIIIa}} \text{PI(1-12)-GEE+ } NH_4^+$$

$$NH_4^+ + \alpha\text{-ketoglurate + NADPH} \xrightarrow{\text{GLDH}} NADP^+ + \text{glutamate} + H_2O$$

COMPOSITION

The TECHNOCHROM® FXIII Kit for photometric FXIII determinations contains:

mL	reagent	other data
3 x 3	FXIII Activator Reagent	Hepes, Thrombin, Polybrene, Fibrin pol. inh tetrapept.
3 x 3	FXIII Detection Reagent	Hepes, GEE, α-ketoglutarate, PI(1-12), ADP, GLDH
3 x 3	FXIII NADPH Solution	NADPH
3 x 1	FXIII Inhibitor Reagent	2-iodoacetamide
1 x 6	Stabilizer Solution	Na-acid

MATERIAL REQUIRED (not supplied with the kit)

- Pipettes
- Distilled water
- Reference Plasma**
  REF 5220110 Coagulation Reference 5 x 1 mL
- Control Plasma Normal and Abnormal**
  REF 5020040 Coagulation Control N 5 x 1 mL
  REF 5021055 Coagulation Control A 5 x 1 mL

** or any other package sizes, special Ceveron® alpha or TECHNOCLOT® Control and Calibration reagents of Technoclone.

WARNING AND PRECAUTIONS

- For research use only
- All blood and plasma samples and products have to be regarded as potentially infectious and handled with appropriate care and in compliance with the biosafety regulations in force and must be disposed of in the same way as hospital waste.
- Each single donor plasma and each lot of Ref. Standards are tested and found negative for Hb_SAg, HIV 1/2 Ab and HCV Ab. However, universal precautions (treating all human source materials as if potentially infectious) should be exercised.
- The Stabilizer solution contains sodium azide. Do not ingest and avoid contact with the skin and mucous membranes. It can be removed with plenty of water. Sodium azide can form explosive azides when contacting heavy metals (e.g. copper or lead)!

STABILITY AND STORAGE

The expiry date printed on the labels applies to storage of the unopened bottles at +2…8 °C.

Stability after reconstitution :

Stability	+2…8°C	-20°C
FXIII Activator Reagent	3 days	3 months
FXIII Detection Reagent	3 days	3 months
FXIII NADPH Solution	3 days	3 months
FXIII Inhibitor Reagent	3 days	3 months

Stability Working Solution	+2…8°C	-20°C
Working Solution	2 days	1 month

TEST PROCEDURE

PREPARATION OF PLASMA SAMPLES

Plasma separation:
Mix 9 parts of venous blood and 1 part sodium citrate solution (0.11 mol/L) and centrifuge for 15 minutes at a RCF of at least 2500 g (corresponding to DIN 58905). The plasma sample can be stored at room temperature up to 24 hours, or at 4°c up to 3 days; otherwise the sample has to be frozen immediately after centrifugation. Stability at –20°C or -70°C is 6 month.

PREPARATION OF REAGENTS

All reagents including distilled water should have reached room temperature before use. The lyophilized reagents are dissolved in the volume of distilled water, NADPH or Stabilizer solutions indicated on the vial and are ready for use after 10 minutes. For standardization test a reconstitution time of 30 min is recommended.

1 FXIII Activator Reagent: DIL 3 mL NADPH 3
2 FXIII Detection Reagent: DIL 3 mL AQUA
3 FXIII NADPH Solution: DIL 3 mL AQUA
4 FXIII Inhibitor Reagent: DIL 1 mL STAB 5
5 FXIII Stabilizer Solution: RTU

WORKING SOLUTION

Prepare the working solution by mixing equal parts of reagent 1 FXIII Activator Reagent and 2 FXIII Detection Reagent and use this mixture for the preparation of sample reagent solution and blank reagent solution.

SAMPLE REAGENT SOLUTION

Add 1part of 5 FXIII Stabilizer solution to 20 parts of the working solution. E.g. add 150µL FXIII Stabilizer Solution to 3 mL working solution.

BLANK REAGENT SOLUTION

Add 1part of 4 FXIII Inhibitor Reagent to 20 parts of the working solution. E.g. add 150µL FXIII Inhibitor Reagent to 3 mL working solution
The Sample Reagent Solution and the Blank Reagent Solution prepared this way are used for the assays.

PERFORMANCE OF THE TEST

Wavelength: 340 nm Measurements are done at + 37°C in temperature controlled spectrophotometer, microplate readers (ELISA) or clinical laboratory analyser.

Pipetting scheme manual method:

Reagent / Solution	Sample	Blank
Plasma	25 µL	25 µL
Sample reagent solution	250 µL	-
Blank reagent solution	-	250 µL
Read absorbance after 5 min. Repeat reading after exactly 2, 4 and 6 min.		

If the assay is adapted to a clinical laboratory analyzer, sample and reagent volumes are proportionally decreased, and the measuring interval is set to 5-10 min.

It is important to use plasma blank to correct for reactions leading to FXIIIa independent NH_3 production and NADPH consumption. Although in most cases such reactions cause only a few percent of alteration in the FXIII activity results, in samples with severe FXIII deficiency the omission of such correction might lead to clinically significant overestimation of FXIII activity.

For each FXIII determination series, the ΔA/min value of the calibration plasma is determined – the result will be used for calculation of the factor (F).

Alternativ, the Coagulation Reference can be used for a calibration. Dilute Coagulation Reference 1:1, 1:2, and 1:4 with NaCl.

APPLICATIONS FOR INSTRUMENTS

Application sheets for auto analyzers are available from Technoclone or your local distributor upon request.

LIMITATIONS OF THE TEST

Bilirubin up to 200 µmol/L and triglycerides up to 7.5 mmol/L do not interfere with the assay. At higher bilirubin or triglyceride concentrations the assay may be repeated with diluted plasma sample.
High concentrations of ammonia (>200 µmol/L) may result in significant NADPH consumption, i.e. the absorbance significantly decreases during the lag phase of the reaction. This may lead to an underestimation of FXIII activity. In these rare cases dilute the plasma sample with physiological saline solution and repeat the assay.

ANALYSES RESULTS

From the absorbance changes during the measuring periods mean ΔA/min values of the sample and the sample blank are calculated. Using automatic kinetic photometers or clinical laboratory analysers mean ΔA/min is directly obtained. FXIII activity can be evaluated by using a factor.

The factor (F) is calculated from the ΔA/min value measured for the calibration plasma and from the assigned FXIII activity of the calibration plasma according to the following formula:

$$F = \frac{FXIII_{cal.}}{\Delta A_{cal.} / min - \Delta A_{cal.\ blank} / min}$$

ΔA/min value of the sample is multiplied by the factor to obtain results as percentage of normal average.

FXIII sample = F x (ΔA sample / min – ΔA sample blank / min)

Alternativ, construct a calibration curve from the ΔA/min value measured for the calibration plasma dilutions and the assigned FXIII activity level of the calibration plasma. Samples are read directly of this curve.

STANDARDIZATION

The Coagulation Reference is calibrated against the FXIII Reference Preparation of the WHO.

REFERENCE RANGE

69 – 143 % of normal

LINEARITY AND DETECTION LIMIT

The method is linear up to 300% FXIII activity.
The detection limit is 0.6%. Please refer to the analyser application for individual detection limit information.

LITERATURE

Please contact Technoclone or your local distributor.