IMMBIOMED
ImmBioMed GmbH & Co. KG
Bergstr. 85, DE-64319 Pfungstadt, Germany
Phone: +49 6157 9799 685 Fax: +49 6157 86443

Fluorescent Factor XIII assay

Product no. ADG866

Storage: 2 – 8°C **For Research Use Only!**

PRODUCT INSERT ***ENGLISH***

INTENDED USE
Determination of Factor XIII activity in plasma samples.

ASSAY PRINCIPLE
Factor XIII is converted by Thrombin into Factor XIIIa. At the same time, Thrombin converts Fibrinogen into Fibrin. The clotting is prevented by an aggregation inhibiting peptide. Factor XIIIa cleaves a dark quenching molecule from the side chain of a modified peptide incorporating glycine methyl ester. Subsequently, the fluorescence of an N-terminal coupled dye increases and can be monitored on-line (excitation wavelength 313 nm; emission wavelength 418 nm). Basically, the isopeptidase activity of Factor XIIIa was described by Parameswaran et al. [1], the modified peptide used is intellectual property of Zedira [2,3].

TEST SAMPLE
Trisodium Citrate anticoagulated plasma

REAGENTS IN THE KIT
(1) SUBSTRATE REAGENT: 2 x 23 µL modified peptide dissolved in DMSO (2) ACTIVATOR REAGENT: 2 x 100 NIH Units human Thrombin, lyophilized (3) BUFFER REAGENT: 2 x TRIS buffer pH 7.5 containing calcium chloride, sodium chloride, poly ethylene glycol (PEG), glycine methyl ester, clot inhibitor peptide, Heparin antagonist (hexadimethrine bromide) and sodium azide, lyophilized.

Reagent preparation, storage and stability
In their original packing box, when stored at 2-8 °C, the unopened reagents are stable until the expiration date printed on the box.
Dissolve one vial of BUFFER REAGENT (3) in 18 mL distilled water.
Dissolve one vial of ACTIVATOR REAGENT (2) in 100 µL of reconstituted BUFFER REAGENT and add the whole volume promptly to the vial of BUFFER REAGENT (3).
Subsequently, add 20 µL of one vial of SUBSTRATE REAGENT (1) and mix thoroughly (REAGENT MIXTURE).

STABILITY OF THE REAGENT MIXTURE AFTER RECONSTITUTION

Temperature	Stability
37 °C	4 hours
20 °C	8 hours
4 °C	1 day
-20 °C	2 months

Protect the REAGENT MIXTURE from light !

EQUIPMENT
The Fluorescent Factor XIII assay can be used in standard fluorescence spectrophotometers. Refer to the instructions of the manufacturer.

SPECIMENS
Blood (9 vol.) must be collected on 0.109 M citrate anticoagulant (1 vol.); plasma supernatant is decanted following a 10 min. centrifugation at 2,500 g; citrated plasma should be tested within 8 hours or stored frozen at -20 °C or below for up to 6 months.

PROCEDURE
Prewarm the REAGENT MIXTURE in suitable fluorescence cuvettes to 37 °C before testing. Add 100 µl of sample to 900 µl of REAGENT MIXTURE, mix thoroughly and start measuring at 37 °C at the following instrument parameters:

Excitation wavelength	313 nm
Emission wavelength	418 nm
Ex. Slit (nm)	5
Em. Slit (nm)	5
Averaging Time (s)	2.0000
Cycle time (min)	0.0000
Stop time (min)	15.0000
Emission filter	Open
Excitation Filter	Auto
PMT voltage (V)	Medium (600 V)

However, these Instrument parameters are recommendations only and should be adjusted to the instrument used to obtain a suitable signal to noise ratio.

RESULTS
The increase in fluorescence after reaching the linear phase (typically period between 5 and 10 minutes after starting the reaction by adding the sample) is proportional to the Factor XIII activity. The results can be evaluated using a reference curve. To calculate the reference curve, STANDARD HUMAN PLASMA is diluted with saline and measured in double determination. The data can be compared to Figure 1 showing a typical plot.

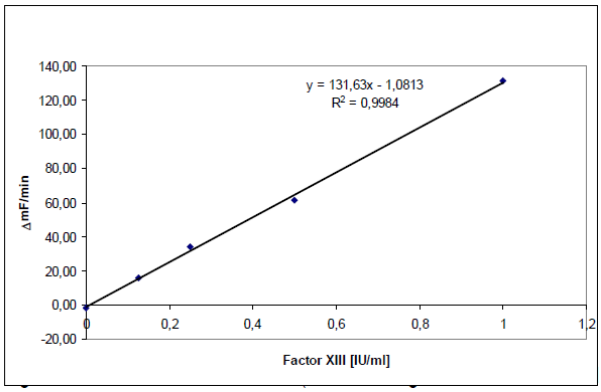

Fig. 1: STANDARD HUMAN PLASMA (calibrated against WHO Standard for Factor XIII) diluted with saline

LIMITATIONS
The use of the FLUORESCENT FACTOR XIII ASSAY should be carefully validated by using suitable CONTROL PLASMAS. Basically, the assay can be performed in a 96 well plate reader. However, it should be noted that the assay is meant for research and development only. It can be feasible varying the sample to REAGENT MIXTURE to improve results.
Very low or very high concentrations of fibrinogen could influence the enzymatic activity of Factor XIII.

REFERENCE RANGE, PRECISION AND SPECIFICITY
The range has been found to be 70 – 140 % of normal. The coefficient of variation in the series was 3.6% for STANDARD HUMAN PLASMA. Day to day variance was 3.6%. No interfering plasma activities are known to the manufacturer.

REFERENCES
1) Parameswaran KN, Cheng XF, Chen EC, Velasco PT, Wilson JH, Lorand L.; Hydrolysis of gamma:epsilon isopeptides by cytosolic transglutaminases and by coagulation factor XIIIa, J Biol Chem. 1997, 10311-7.

2) Oertel K and Pasternack R; Fluorescence-based kinetic determination of the activity of Transglutaminases (EP04019090.2; US60/603,374).

3) Oertel K, Hunfeld A, Specker E, Reiff C, Seitz R, Pasternack R, Dodt J; A highly sensitive fluorometric assay for determination of human coagulation factor XIII in plasma, Analytical Biochemistry 2007, 152-158.

ADG866©25062019

Hinweis/Note:
Der Packungsbeileger dient nur als erste Information. Der relevante Packungsbeileger liegt der Ware bei.

The datasheet is for information purposes only. The current datasheet will be enclosed with product shipment.