Human Factor XIII ELISA Kit

Enzyme Immunoassay for the quantification of Factor XIII in human plasma, serum, milk, urine, saliva, and cell culture samples

Catalog number: ARG81134

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INTRODUCTION

This gene encodes the coagulation factor XIII A subunit. Coagulation factor XIII is the last zymogen to become activated in the blood coagulation cascade. Plasma factor XIII is a heterotetramer composed of 2 A subunits and 2 B subunits. The A subunits have catalytic function, and the B subunits do not have enzymatic activity and may serve as plasma carrier molecules. Platelet factor XIII is comprised only of 2 A subunits, which are identical to those of plasma origin. Upon cleavage of the activation peptide by thrombin and in the presence of calcium ion, the plasma factor XIII dissociates its B subunits and yields the same active enzyme, factor XIIIa, as platelet factor XIII. This enzyme acts as a transglutaminase to catalyze the formation of gamma-glutamyl-epsilon-lysine crosslinking between fibrin molecules, thus stabilizing the fibrin clot. It also crosslinks alpha-2-plasmin inhibitor, or fibronectin, to the alpha chains of fibrin. Factor XIII deficiency is classified into two categories: type I deficiency, characterized by the lack of both the A and B subunits; and type II deficiency, characterized by the lack of the A subunit alone. These defects can result in a lifelong bleeding tendency, defective wound healing, and habitual abortion. [provided by RefSeq, Jul 2008]

PRINCIPLE OF THE ASSAY

This is an Enzyme Immunoassay for the quantification of Human Factor XIII in plasma, serum, milk, urine, saliva, and cell culture samples.

This assay employs the sandwich enzyme immunoassay technique. A mouse antibody specific for Factor XIII has been pre-coated onto a microtiter plate. Factor XIII in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for Factor XIII. The biotinylated antibody interacts with streptavidin-horseradish peroxidase (SA-HRP) which

catalyzes the substrate solution. The reaction is monitored by a color change which is readable at OD of 450 nm±2 nm. Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standards. The intensity of color development is proportional to the amount of Factor XIII in the samples.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the kit as Storage information below. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated Microtiter Plate	12 x 8 wells	4°C
Human FXIII Standard	1 vial (320 ng, lyophilized)	4°C (store at -20°C after reconstitution)
50X Biotinylated Human Factor XIII antibody concentrate	1 vial (120 µl)	-20°C
100X Streptavidin-HRP concentrate	80 µl	-20°C
10X EIA Diluent concentrate	30 ml	4°C
20X wash buffer concentrate	30 ml X 2 bottles	4°C
TMB Substrate	8 ml	4°C, ready for use
STOP solution	12 ml	4°C, ready for use
Plate sealer	3 pieces	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the components at recommended temperature.
- Briefly spin down the standards and solutions before use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- If crystals are observed in the 20X Wash buffer or 10X EIA Diluent, warm to RT and mix gently until the crystals are completely dissolved.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines.

Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect supernatants. Dilute samples 1000X into 1X EIA Diluent and assay (Dilution factor=1000). The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).

Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes, and collect serum. Dilute samples 1000X into 1X EIA Diluent and assay (Dilution factor=1000). The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Dilution Note (For duplicate):

a) For 1:100 dilution: add 5 µL of samples into 495 µL of 1X EIA Diluent, mix well.

b) For 1:1000 dilution: add 20 µL of diluted samples from a) into 180 µL of 1X EIA Diluent, mix well.

Cell Culture Supernatants: Collect cell culture media and centrifuge at 1500 rpm for 10 minutes at 4°C to remove debris. Collect supernatants and assay. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

Urine: Collect urine using sample tube. Centrifuge samples at 800 x g for 10 minutes. It is suggested dilute samples 1:2 with 1X EIA Diluent and assay immediately (Dilution factor=2. E.g. 100 µl of sample + 100 µl of 1X EIA Diluent, user should determine optimal dilution factor depending on application needs). The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Dilution before assay maybe not necessary, however, user should determine optimal dilution factor depending on application needs.

Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. It is suggested dilute samples 1:4 with 1X EIA Diluent and assay immediately (Dilution factor=4. E.g. 50 µl of sample + 150 µl of 1X EIA Diluent, user should determine optimal dilution factor depending on application needs). The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X EIA Diluent: Dilute 10X EIA Diluent into distilled water to yield 1X EIA Diluent (E.g. 10 ml of 10X EIA Diluent + 90 ml of distilled water). If crystals appear in buffer, warm the buffer in warm water bath for 30 minutes or mix gently until crystals disappear. Mix well before use. Diluted 1X EIA Diluent can be stored for up to 30 days at 2-8°C.
- Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. If crystals appear in buffer, warm the buffer in warm water bath for 30 minutes or mix gently until crystals disappear. Mix well before use.
- Biotinylated Human Factor XIII antibody: Briefly spin down the 50X Biotinylated Human Factor XIII antibody. Dilute the antibody with 1X EIA Diluent buffer (E.g. 60 µl of antibody + 2940 µl of 1X EIA Diluent). Any remaining solution should be frozen at -20°C.
- Streptavidin-HRP conjugate: Spin down the Streptavidin-HRP Conjugate (100X) briefly and dilute the desired amount of the conjugate with 1X EIA Diluent (E.g. 40 µl of Streptavidin-HRP conjugate + 3960 µl of 1X EIA Diluent). Any remaining solution should be frozen at -20°C.

- **Standard peptide**: Reconstitute the Standard vial (320 ng) with 2 ml of 1X EIA Diluent. The concentration of this stock solution is 160 ng/ml. Allow it to sit for 10 minutes at RT with gentle agitation prior to making dilutions. Mix well and spin down before use. Dilute standard solutions according to the table below and make serial dilutions of 160 ng/ml, 80 ng/ml, 40 ng/ml, 20 ng/ml, 10 ng/ml, 5 ng/ml and 2.5 ng/ml. And the 1X EIA Diluent serves as the zero standard. Any remaining stock solution should be stored at -20°C and used within 30 days. Aliquot to avoid repeated freeze-thaw cycles is recommended.

Standard No.	Standard	1X EIA Buffer	Concentrations (ng/ml)
S1	400 µl	0 µl	160
S2	200 µl of S1	200 µl	80
S3	200 µl of S2	200 µl	40
S4	200 µl of S3	200 µl	20
S5	200 µl of S4	200 µl	10
S6	200 µl of S5	200 µl	5
S7	200 µl of S6	200 µl	2.5
B	0	200 µl	0

ASSAY PROCEDURE

1. Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator. The remaining microplate strips may be stored for up to 30 days in a vacuum desiccator.
3. Standards, samples and controls should be assayed in duplicates.
4. Add 50 µl of Human FXIII Standard or sample per well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed.
5. Cover wells with a sealing tape and incubate for 2 hours at room temperature (20-25°C). Start the timer after the last addition.
6. Remove sealer from plate.
7. Aspirate each well and wash, repeating the process 4 times for a total 5 washes (If a microplate washer is used, wash the wells for a total 6 washes). Wash by filling each well with 1× Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
8. Add 50 µl Biotinylated Human FXIII antibody into each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have

formed.

9. Reseal the plate with sealer. Incubate for 1 hour at RT.
10. Wash as according to step 7.
11. Add 50 µl Streptavidin-HRP solution into each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed.
12. Reseal the plate with sealer. Incubate for 30 minutes at RT. (Turn on the microplate reader and set up the program in advance.)
13. Wash as according to step 7.
14. Add 50 µl TMB substrate solution into each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed.
15. Incubate for 10 minutes at RT or until the optimal blue color density develops. (Protect from light)
16. Add 50 µl STOP solution into all wells to stop the reaction. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
17. Read the OD with a microplate reader at 450 nm **immediately.** If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

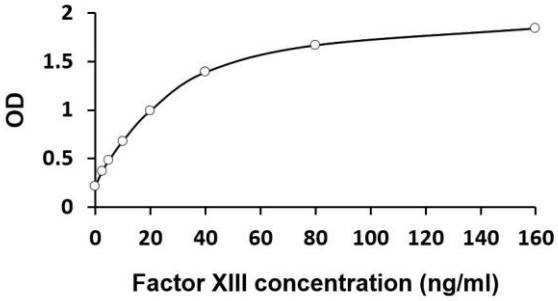
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



Reference Values:

Normal human factor XIII plasma levels range from 10 to 20 µg/ml.

Human plasma and serum samples from healthy adults were tested (n=40).

On average, factor XIII level was 14.8 µg/ml.

QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of human FXIII as calculated by 2SD from the mean of a zero standard was established to be 2.1 ng/ml.

Standard Range

Standard Range: 2.5- 160 ng/ml

Recovery

92-113%

Linearity

Sample Dilution	Plasma	Serum
500x	95%	98%
1000x	101%	101%
2000x	104%	104%

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 4.4 % and inter-assay precision was 9.4 %.

Cross Reactivity

The kit cross-reacts:

FXIII (Human) 100%

FXIIIa (Human) 100%

FXIII (Monkey) < 40%

This kit detects no cross-reactivity with the following factor:

FXIII (Bovine)

FXIII (Canine)

FXIII (Mouse)

FXIII (Rabbit)

FXIII (Rat)

FXIII (Swine)