AssayMax™

Human Factor XIII ELISA Kit

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For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

Assay Summary

Step 1. Add 50 µl of Standard or Sample per well.
Incubate 2 hours.

Step 2. Wash, then add 50 µl of Biotinylated Antibody per well.
Incubate 1 hour.

Step 3. Wash, then add 50 µl of SP Conjugate per well.
Incubate 30 minutes.

Step 4. Wash, then add 50 µl of Chromogen Substrate per well.
Incubate 10 minutes.

Step 5. Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Symbol Key

Consult instructions for use.

Assay Template

	A	B	C	D	E	F	G	H
1								
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Human Factor XIII ELISA Kit

Catalog No. EF1013-1

Sample insert for reference use only

Introduction

Factor XIII is a proenzyme for a plasma transglutaminase previously known as fibrin stabilizing factor. Intracellular FXIII exists as a dimer of two FXIIIa molecules, whereas the circulating plasma FXIII is composed of two FXIIIa and two FXIIIb subunits (1). This tetramer is activated in the presence of thrombin and Ca^{2+} by separation of the two subunits and cleavage of the 37 amino acid activation peptide from the N-terminal of the FXIIIa molecule (2).

Principle of the Assay

The AssayMax Human Factor XIII ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human factor XIII in **plasma, serum, milk, urine, saliva, and cell culture samples**. This assay employs a quantitative **sandwich enzyme immunoassay** technique that measures FXIII in less than 4 hours. A murine antibody specific for FXIII has been pre-coated onto a 96-well microplate with removable strips. FXIII in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for FXIII, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This product is for **Research Use Only** and is Not For Use In Diagnostic Procedures.
- Prepare all reagents (working diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.
- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
- Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.
- The Stop Solution is an acidic solution.
- The kit should not be used beyond the expiration date.

Reagents

- **Human FXIII Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a murine antibody against FXIII.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human FXIII Standard:** Human FXIII in a buffered protein base (160 ng, lyophilized).
- **Biotinylated Human FXIII Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against FXIII (140 µl).
- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrated (80 µl).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- 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.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Other Supplies required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation, and Storage

- **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 1:1000 into EIA Diluent and assay. 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 remove serum. Dilute samples 1:1000 into EIA Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Collect cell culture media and centrifuge at 3000 x *g* for 10 minutes at 4°C to remove debris. Collect supernatants and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:2 into EIA Diluent and assay. 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.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:4 into EIA Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Refer to Sample Dilution Guidelines below for further instruction.

Guidelines for Dilutions of 1:100 or Greater (*for reference only; please follow the insert for specific dilution suggested)*	
1:100	**1:10000**
A) 4 ul sample: 396 µl buffer(100x) = 100 fold dilution *Assuming the needed volume is less than or equal to 400 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) = 10000 fold dilution *Assuming the needed volume is less than or equal to 400 µl.*
1:1000	**1:100000**
A) 4 µl sample : 396 µl buffer (100x) B) 24 µl of A : 216 µl buffer (10x) = 1000 fold dilution *Assuming the needed volume is less than or equal to 240 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) C) 24 µl of B : 216 µl buffer (10x) = 100000 fold dilution *Assuming the needed volume is less than or equal to 240 µl.*

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **EIA Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Human FXIII Standard:** Reconstitute the 160 ng of Human FXIII Standard with 1 ml of EIA Diluent to generate a 160 ng/ml standard stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard stock solution (160 ng/ml) 1:2 with equal volume of EIA Diluent to produce 80, 40, 20, 10, 5, and 2.5 ng/ml solutions. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[FXIII] (ng/ml)
P1	1 part Standard (160 ng/ml)	160.0
P2	1 part P1 + 1 part EIA Diluent	80.00
P3	1 part P2 + 1 part EIA Diluent	40.00
P4	1 part P3 + 1 part EIA Diluent	20.00
P5	1 part P4 + 1 part EIA Diluent	10.00
P6	1 part P5 + 1 part EIA Diluent	5.000
P7	1 part P6 + 1 part EIA Diluent	2.500
P8	EIA Diluent	0.000

- **Biotinylated Human FXIII Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with EIA Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- 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).
- 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.
- Add 50 µl of Human FXIII Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 µl of Biotinylated Human FXIII Antibody to each well and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for 10 minutes or till the optimal color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 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.

Data Analysis

- Calculate the mean value of the duplicate or triplicate for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

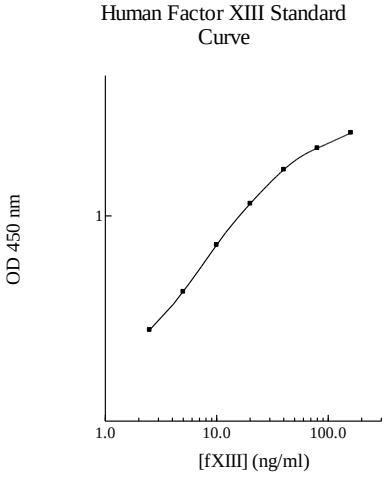
Typical Data

- The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	ng/ml	OD	Average OD
P1	160.0	1.949 1.886	1.917
P2	80.00	1.670 1.605	1.637
P3	40.00	1.433 1.439	1.436
P4	20.00	1.042 1.078	1.060
P5	10.00	0.814 0.779	0.797
P6	5.000	0.545 0.560	0.552
P7	2.500	0.405 0.414	0.409
P8	0.000	0.205 0.208	0.206
Sample: Pool Normal, Sodium Citrate Plasma (1000x)		0.865 0.887	0.876

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Reference Value

- 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 11.2 µg/ml.

Performance Characteristics

- The minimum detectable dose of factor XIII as calculated by 2SD from the mean of a zero standard was established to be 2.5 ng/ml.
- Intra-assay precision was determined by testing replicates of three plasma samples in one assay.
- Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision			**Inter-Assay Precision**		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	4.9%	4.6%	4.0%	9.6%	8.8%	8.2%
Average CV (%)	4.5%			8.9%		

Recovery

Standard Added Value	5 – 80 ng/ml
Recovery %	91 – 110%
Average Recovery %	98%

Linearity

- Plasma and serum samples were serially-diluted to test for linearity.

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
1:500	101%	97%
1:1000	99%	100%
1:2000	101%	104%

Cross-Reactivity

Species	Cross Reactivity (%)
Beagle	None
Bovine	None
Monkey	<40%
Mouse	None
Rat	None
Swine	None
Human Factor XIII	100%
Human Factor XIIIa	100%

References

(1) Schwatz, M.L. *et al.* (1973) *J. Biol. Chem*. 248:1395
(2) Takagi, T. *et al*. (1974) *Biochemistry* 13:750

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