Human Factor XIII (FXIII) ELISA Kit

Introduction

Factor XIII is a proenzyme for a plasma transglutaminase previous 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). Inherited factor XIII deficiency can result from mutations in either the A- or B- subunit genes (3). Factor XIIIA subunit deficiency is an autosomal recessive disorder that is characterized by a life-long bleeding tendency and complications in wound healing (4).

Principal of the Assay

The Human Factor XIII (FXIII) ELISA kit is designed for detection of human factor XIII in plasma, serum, urine and cell culture supernatants. 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 kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **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 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **FXIII Standard:** Human FXIII in a buffered protein base (160 ng, lyophilized).
- **Biotinylated FXIII Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against FXIII (80 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).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrated (90 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

- Store kit at 2-8^{0}C or -20^{0}C upon arrival up to the expiration date.
- Opened EIA Diluent may be stored for up to 1 month at 2-8^{0}C. Store reconstituted reagents at -20^{0}C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, and multiple channel pipettes).
- 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 2000 x g for 10 minutes and collect supernatants. Dilute samples 1:1000 into EIA Diluent and assay as follows: add 10 µl of sample to 990 µl of EIA Diluent (1:100) to make Solution A; then add 50 µl of Solution A to 450 µl of EIA Diluent (1:10) to make a final working solution (1:1000). The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant.)
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:10000 into EIA Diluent and assay as follows: add 10 µl of sample to 990 µl of EIA Diluent (1:100) to make Solution A; then add 50 µl of Solution A to 450 µl of EIA Diluent (1:10) to make a final working solution (1:1000). The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Collect cell culture media and centrifuge at 2000 x g for 10 minutes at 4^{0}C to remove debris. Collect supernatants and assay. The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample tube. Centrifuge samples at 600 x g for 10 minutes and assay. Dilute samples 1:2 into EIA Diluent. Store samples at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Saliva:** Collect saliva using sample tube. Centrifuge samples at 600 x g for 10 minutes and assay. Store samples at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

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

Standard Point	Dilution	[FXIII] (ng/ml)
P1	1 part Standard (160 ng/ml)	160.00
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 FXIII Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with EIA Diluent. Any remaining solution should be frozen at -20^{0}C.
- **Wash Buffer Concentrate (20x):** 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^{0}C.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30^{0}C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer. Invert the plate and decant the contents, and hit the plate 4-5 times on absorbent paper towel to complete remove liquid at each step.
- Add 50 µl of Biotinylated FXIII Antibody to each well and incubate for 1 hour.
- Wash five times with 200 µl of Wash Buffer as 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 five times with 200 µl of Wash Buffer as above.

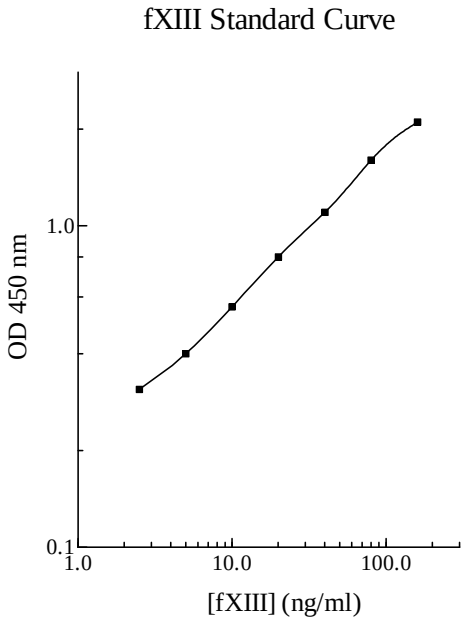
- Add 50 µl of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. 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 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.

Standard Curve

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



Performance Characteristics

- The minimum detectable level of fXIII was typically less than 50 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 5.6% and 6.8% respectively.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:500	102%	97%
1:1000	98%	101%
1:2000	101%	99%

	Average Percentage of Expected Value
Sample Dilution	**Urine**
No Dilution	106%
1:2	98%
1:4	93%

Recovery

Standard Added Value	5 – 50 ng
Recovery %	88-110 %
Average Recovery %	99 %

Cross-Reactivity

Species	% Cross Reactivity
Beagle	< 2
Bovine	None
Monkey	<40 (suggest dilution 1:100 for plasma/serum)
Mouse	< 1
Rat	< 1
Swine	< 1

References

(1) Schwatz, M.L. *et al.* (1973) *J. Biol. Chem.* 248:1395
(2) Takagi, T. *et al.* (1974) *Biochemistry* 13:750
(3) Kangsadalampai, S. *et al.* (1998) *Blood* 92:481
(4) Anwar, R. *et al.* (1998) *Blood* 91:149

Version 4.4