AssayMax Human Factor XIII (FXIII) ELISA Kit

Catalog Number EF1013-1
Lot #

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 AssayMax Human Factor XIII (FXIII) ELISA kit is designed for detection of human factor XIII in plasma and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures FXIII in 3.5 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 (24 ng, lyophilized).
- **Biotinylated FXIII Antibody (100x):** A 100-fold biotinylated polyclonal antibody against FXIII (80 µl).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (120 µl)
- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (10x)**: A 10-fold concentrated buffered surfactant (2 x 30 ml).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydroxychloric acid (12 ml) to stop the chromogen substrate reaction.

Storage Condition

- Store unopened kit at 2 - 8^{0}C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2 - 8^{0}C. Store reconstituted standard 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 2,000x g for 10 minutes and assay. Dilute samples 1:10,000 into EIA Diluent as follows: add 10 µl of sample to 990 µl of EIA Diluent (1:100) to make Solution A; then add 10 µl of Solution A to 990 µl of EIA Diluent (1:100) to make a final working solution (1:10,000). 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 2,000 x g for 10 minutes at 4^{0}C to remove debris. Collect supernatants and assay. The samples can be stored at -20^{0}C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **FXIII Standard:** Reconstitute the 24 ng of human FXIII Standard with 2 ml of EIA Diluent to generate a 12 ng/ml of stock solution. 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 (12 ng/ml) twofold with equal volume of EIA Diluent to produce 6, 3, 1.5, 0.75, 0.375 and 0.188 ng/ml. EIA Diluent serves as the zero standard (0 ng/ml).

Standard Point	Dilution	[FXIII] (ng/ml)
P1	1 part Standard (12 ng/ml) + 1 part EIA Diluent	6.000
P2	1 part P1 + 1 part EIA Diluent	3.000
P3	1 part P2 + 1 part EIA Diluent	1.500
P4	1 part P3 + 1 part EIA Diluent	0.750
P5	1 part P4 + 1 part EIA Diluent	0.375
P6	1 part P5 + 1 part EIA Diluent	0.188
P7	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.
- **EIA Diluent Concentrate (10x):** Dilute the EIA Diluent 1:10 with reagent grade water.
- **Wash Buffer Concentrate (10x):** Dilute the Wash Buffer Concentrate 1:10 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.

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 and incubate for 2 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 30 minutes.
- 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 5 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.

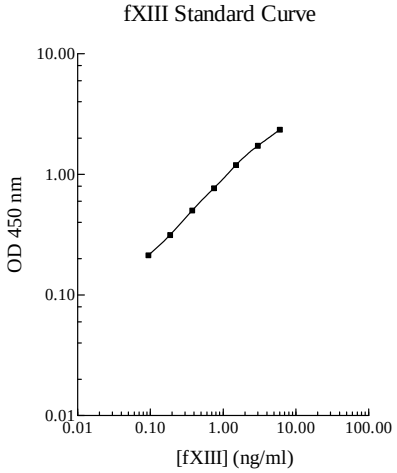
Data Analysis

- 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 of the linear portion using log-log curve fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the plasma value by the dilution factor of 10,000.

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 5.8% and 6.7% respectively.
- No significant cross-reactivity or interference was observed.

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

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