AssayMax Human Factor X (FX) ELISA Kit

Catalog Number EF1010-1

Introduction

Factor X (FX) is a plasma serine protease zymogen involved in the blood coagulation cascade. FX is purified from plasma as a two-chain protein consisting of a 45 kDa heavy chain and a 17 kDa light chain. The FX heavy chain is cleaved during coagulation by several different proteases including the intrinsic Xase complex, the FX-activating enzyme from Russell's viper venom (RVV) and trypsin, and also by extrinsic (tissue factor/factor VIIa) pathway to give an active enzyme FXa. FXa as the activator of prothrombin occupies a central position linking the two blood coagulation pathways (1 - 4).

Principal of the assay

The AssayMax Human Factor X (FX) ELISA kit is designed for detection of human factor X in plasma and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures FX in 3.5 hours. A polyclonal antibody specific for FX has been pre-coated onto a 96-well microplate with removable strips. FX in standards and samples is sandwiched by the immobilized antibody and the peroxidase conjugated polyclonal antibody specific for FX. 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

- **FX Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human FX.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **FX Standard:** Plasma human FX in a buffered protein base (400 ng, lyophilized).
- **Peroxidase Conjugated FX Antibody (100 x concentrate):** A Peroxidase Conjugated polyclonal antibody against FX (80 µl).

- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (20 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. Standard, antibody and enzyme conjugate must be kept at < **-20^{0}C** separately**.**
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal or using a pouch sealer. Store 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)
- Deionized or distilled reagent grade water
- Incubator (37^{0}C)

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using 3.8% sodium citrate as an anticoagulant. Centrifuge samples at 2,000x g for 10 minutes and assay. Samples may require 1:200 dilution into EIA Diluent. Store samples at <-20 ^{0}C. 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. Store samples at < -20 ^{0}C. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **Standard Curve:** Reconstitute the 400 ng of human FX Standard with 2 ml of EIA Diluent to generate a stock solution of 200 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 (200 ng/ml) twofold with equal volume of EIA Diluent to produce 100, 50, 25, 12.5, 6.25 and 3.125 ng/ml. EIA Diluent serves as the zero standard (0 ng/ml).

Standard Point	Dilution	[FX] (ng/ml)
P1	1 part Standard (200 ng/ml)	200.000
P2	1 part P1 + 1 part EIA Diluent	100.000
P3	1 part P2 + 1 part EIA Diluent	50.000
P4	1 part P3 + 1 part EIA Diluent	25.000
P5	1 part P4 + 1 part EIA Diluent	12.500
P6	1 part P5 + 1 part EIA Diluent	6.250
P7	1 part P6 + 1 part EIA Diluent	3.125
P8	EIA Diluent	0.000

- **Peroxidase Conjugated FX 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.

Assay Procedure

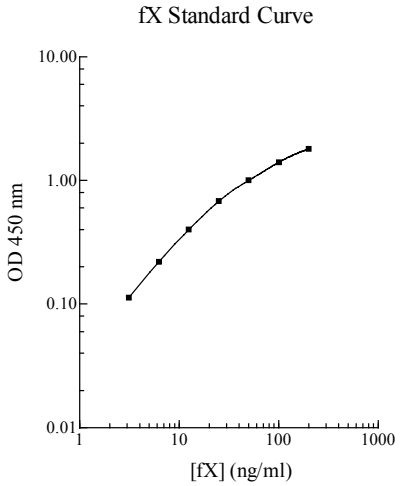
- Prepare all reagents, working standards and samples as instructed.
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Standard or sample per well. Cover wells and incubate for 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 it 4-5 times on absorbent paper towel to complete remove liquid at each step.
- Add 50 µl of Peroxidase Conjugated FX Antibody to each well and incubate for 60 minutes.
- Wash five times with 200 µl of Wash Buffer as above.
- Add 50 µl of Chromogen Substrate per well and incubate for approximately 10 minutes or till the optimal blue color density develop. 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 readings 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 of the log-log curve.
- Determine the unknown sample concentration from the Standard Curve and multiply the plasma or tissue value by the dilution factor of 200.

Standard Curve

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



Sensitivity and Specificity

- The minimum detectable dose of human FX is typically < 3 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.4 % and 7.7% respectively.
- No significant cross-reactivity or interference was observed.

References

1. Ruf, W. and Edgington, T.S. (1994) *FASEB J*. 8:385
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3. Messier, T.L. *et al*. (1991) *Gene* 99:291
4. Di Scipio, R.G. *et al.* (1977) Biochemistry 16:5253

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