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 4 hours. A monoclonal 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 monoclonal 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 (1.2 µg, lyophilized).
- **Biotinylated FX Antibody (100x):** A 100-fold biotinylated polyclonal antibody against H. FX (80 µl).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (90 µl).
- **MIX Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml).

- **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 MIX 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, 200-1000 µl and multiple channel)
- Deionized or distilled reagent grade water

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using 3.8% sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Samples may require 1:800 dilution into MIX Diluent. 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).
- **Cell Culture Supernatants:** Collect cell culture media and centrifuge at 2000 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. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **MIX Diluent Concentrate (10x):** Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8^{0}C.
- **Standard Curve:** Reconstitute the 1.2 µg of human FX Standard with 3 ml of MIX Diluent to generate a Stock solution of 400 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by diluting the Stock solution (400 ng/ml) 1:4 with MIX Diluent to generate 100ng/ml. Serially dilute twofold with equal volume of MIX Diluent to produce 50, 25, 12.5, 6.25, 3.125 and 1.563 ng/ml. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20^{0}C.

Standard Point	Dilution	[FX] (ng/ml)
P1	1 part Stock (400 ng/ml) + 3 part MIX Diluent	100.000
P2	1 part P1 + 1 part MIX Diluent	50.000
P3	1 part P2 + 1 part MIX Diluent	25.000
P4	1 part P3 + 1 part MIX Diluent	12.500
P5	1 part P4 + 1 part MIX Diluent	6.250
P6	1 part P5 + 1 part MIX Diluent	3.125
P7	1 part P6 + 1 part MIX Diluent	1.563
P8	MIX Diluent	0.000

- **Biotinylated FX Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent. Any remaining solution should be frozen at -20^0C.
- **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 MIX Diluent. Any remaining solution should be frozen at -20^0C.

Assay Procedure

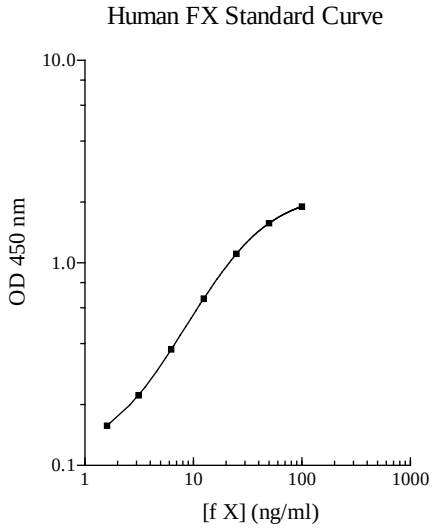
- Prepare all reagents, working standards and samples as instructed.
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl 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 Biotinylated FX Antibody to each well and incubate for 1 hour.
- Wash five times with 200 µl of Wash Buffer.
- 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 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 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 used for illustration only. A standard curve should be generated each time the assay is performed.



Precision, Sensitivity and Specificity

- The minimum detectable dose of human FX is typically < 1 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.8 % and 7.1% respectively.

Linearity

	Average Percentage of Expected Value
Sample Dilution	**Plasma**
1:800	102%
1:1600	97%
1:3200	100%

Recovery

Standard Added Value	5 – 50 ng/ml
Recovery %	82-117 %
Average Recovery %	99.5 %

Cross-Reactivity

- No significant cross-reactivity or interference was observed.

References

(1) Ruf, W. and Edgington, T.S. (1994) *FASEB J*. 8:385
(2) Neuenschwander, P.F. *et al.* (1993) *Thrombosis and Haemostasis* 70:970
(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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