



AssaySense Human Factor X (FX) Chromogenic Activity Assay Kit

Catalog No. CF1010

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).

Principle of Assay

The AssaySense Human FX Chromogenic Activity Assay Kit is developed to determine human FX activity in plasma and cell culture. The assay measures the activation of zymogen FX to FXa by RVV. The amidolytic activity of the FXa is quantitated using a highly specific FXa substrate releasing a yellow para-nitroaniline (pNA) chromophore. The change in absorbance of the pNA at 405 nm is directly proportional to the FX enzymatic activity.

Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- All human source materials have been tested and found to be negative to HbsAg, HIV-1 and HCV by FDA approved methods.

Reagents

The activity assay kit contains sufficient reagents to perform 100 tests using microplate method.

- **Microplate:** One 96-well polystyrene microplate (12 strips of 8 wells)
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Sample Diluent (6x):** A six-fold concentrate (10 ml)
- **Human FX Standard:** 1 vial (10 µg)
- **Assay Diluent:** A working solution (10 ml)
- **RVV:** 1 vial
- **FXa Substrate:** 2 vials

Storage Condition

- Store kit at -20°C upon arrival up to the expiration date.
- Opened reagents may be stored for up to 1 month at -20°C.
- Store reconstituted standard and reagents at -20°C or below.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 405 nm
- Pipettes (1-20 µl, 20-200 µl, and multiple channel)
- Deionized or distilled reagent grade water
- Incubator (37°C)

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 assay. Dilute samples 1:10 with Sample Diluent and assay immediately. Samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x g for 15 minutes at 4°C to remove debris. Collect supernatants and assay.

Reagent Preparation

- **Sample Diluent:** Dilute Sample Diluent Concentrate 1:6 with reagent grade water.
- **Standard Curve:** Reconstitute the FX Standard (10 µg) with 1.25 ml of Sample Diluent to generate a stock solution of 8 µg/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions.
 - For high level of FX standard, prepare duplicate or triplicate standard points by serially diluting the standard solution with equal volume of Sample Diluent to produce 4, 2, 1, 0.5, 0.25 and 0.125 µg/ml solutions. Sample Diluent serves as the zero standard (µg/ml). Any remaining solution should be frozen at -20°C.
 - For low level of FX standard, dilute stock solution (8 µg/ml) 1:8 with Sample Diluent to produce 1 µg/ml standard. Prepare duplicate or triplicate standard points by serially diluting the standard solution 1:4 with equal volume of Sample Diluent to produce 0.25, 0.0625 and 0.0156 µg/ml solutions. Sample Diluent serves as the zero standard (µg/ml). Any remaining solution should be frozen at -20°C.

Standard curve for high level of FX activity samples:

Standard Point	Dilution	[FX] (µg/ml)
P1	1 part Standard (8 µg/ml)	8.000
P2	1 part P1 + 1 part Sample Diluent	4.000
P3	1 part P2 + 1 part Sample Diluent	2.000
P4	1 part P3 + 1 part Sample Diluent	1.000
P5	1 part P4 + 1 part Sample Diluent	0.500
P6	1 part P5 + 1 part Sample Diluent	0.250
P7	1 part P6 + 1 part Sample Diluent	0.125
P8	Sample Diluent	0.000

Standard curve for low level of FX activity samples:

Standard Point	Dilution	[FX] (µg/ml)
P1	1 part P1 + 7 parts Sample Diluent	1.000
P2	1 part P1 + 3 parts Sample Diluent	0.250
P3	1 part P2 + 3 parts Sample Diluent	0.0625
P4	1 part P3 + 3 parts Sample Diluent	0.0156
P5	Sample Diluent	0.000

- **RVV:** Add 1.2 ml of Sample Diluent. Allow the RVV to sit for 10 minutes to dissolve.
- **FXa Substrate:** Add 1.1 ml of reagent grade water. Allow the substrate to sit for 10 minutes to dissolve.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at 37 °C for chromogenic activity assay. Seal the plate with sealing tape at each step.
- Remove excess microplate strips from the plate frame.
- Add 20 µl of Factor X Standard or diluted sample to each well.
- Assay Mix: At room temperature, freshly prepare the desired volume of the Mix by combining the following reagents according to the assay numbers (n) plus one.

Reagents	n=1
Assay Diluent	10 µl
RVV	10 µl
FXa Substrate	20 µl

- Add 40 µl of above Assay Mix to each well. Read the absorbance at 405 nm at zero minutes for background O.D. Seal the plate with sealing tape and incubate at 37 °C.
 - For high level of FX activity, read the absorbance every minute for 4 minutes.
 - For low level of FX activity, read the absorbance every 5 minutes for 40 minutes.

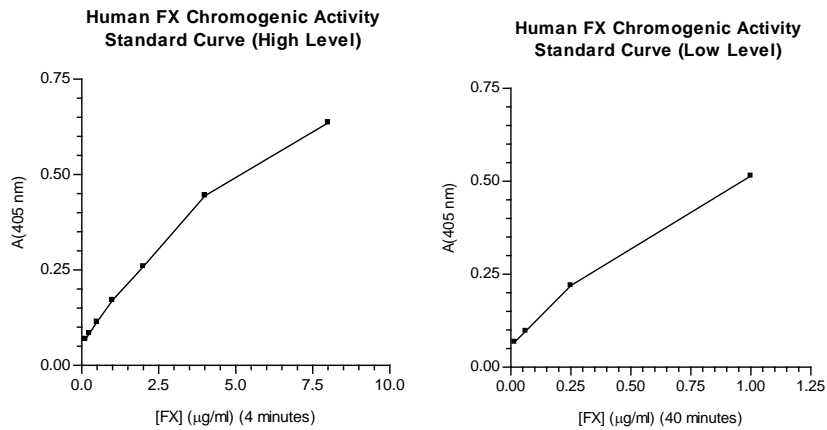
FX or Samples	20 µl
Assay Mix	40 µl
High FX activity Samples: Incubate 37°C, read absorbance at 405 nm every minute for 4 minutes	
Low FX activity Samples: Incubate 37°C, read absorbance at 405 nm every 5 minutes for 40 minutes	

Data Analysis

- Calculate the mean value of the duplicate or triplicate for each standard and sample.
- To generate a Standard Curve from the initial reaction time, plot the graph using the standard concentrations on the x-axis and the corresponding mean 405 nm absorbance or change in absorbance per minute (ΔA/min) on the y-axis. The best-fit line can be determined by regression analysis of the linear portion of the curve.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

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



Performance Characteristics

- Heparin concentration below 30 U/ml does not interfere with the assay.
- No other enzyme that activates the substrate in plasma 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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