

## **AssaySense Human Factor X (FX) Chromogenic Activity Assay Kit**

Catalog Number CF1010

Lot

### **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):** 10 ml
- **Assay Diluent:** 10 ml

- **RVV:** 1 vial
- **Human FX Standard:** 1 vial (10 µg)
- **FXa Substrate:** 2 vials

## Storage Condition

- Store unopened kit at 2-8<sup>0</sup>C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8<sup>0</sup>C. Store reconstituted standard and reagents at -20<sup>0</sup>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<sup>0</sup>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 3,000x g for 10 minutes and assay. Dilute samples 1:10 with Sample Diluent and assay immediately. Samples can be stored at -20<sup>0</sup>C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3,000x g for 15 minutes at 4<sup>0</sup>C to remove debris. Collect supernatants and assay.

## Reagent Preparation

- **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 standard points by serially diluting the standard solution twofold 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).

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 standard points by serially diluting the standard solution 1:4 with equal volume of Sample Diluent to produce 0.25, 0.0625, 0.01563, 0.0039, and 0.00098 µg/ml solutions. Sample Diluent serves as the zero standard (µg/ml).

**Standard curve for high level of FX activity samples:**

Standard Point	Dilution	[FX] (µg/ml)
P1	1 part Standard	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 Standard	1.00000
P2	1 part P1 + 3 part Sample Diluent	0.25000
P3	1 part P2 + 3 part Sample Diluent	0.06250
P4	1 part P3 + 3 part Sample Diluent	0.01563
P5	1 part P4 + 3 part Sample Diluent	0.00390
P6	1 part P5 + 3 part Sample Diluent	0.00098
P7	Sample Diluent	0.00000

- **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**

- Add 20 µl of Factor X Standard or diluted sample to the microplate.
- Assay Mix: At room temperature, freshly prepare the desired volume of the Mix by combining the following reagents according to the assay numbers (n).

<u>Reagents</u>	<u>n=1</u>
Assay Diluent	10 µl
RVV	10 µl
FXa Substrate	20 µl
- Add 40 µl of above Assay Mix to each well of the 96-well plate.
- Incubate at 37 °C and read the absorbance at 405 nm. For high level of FX activity, read the absorbance every 2 minutes and the results usually can be achieved between 2 and 10 minutes. For low level of FX activity, read the absorbance every 10 minutes and the results usually can be achieved from 20 to 60 minutes

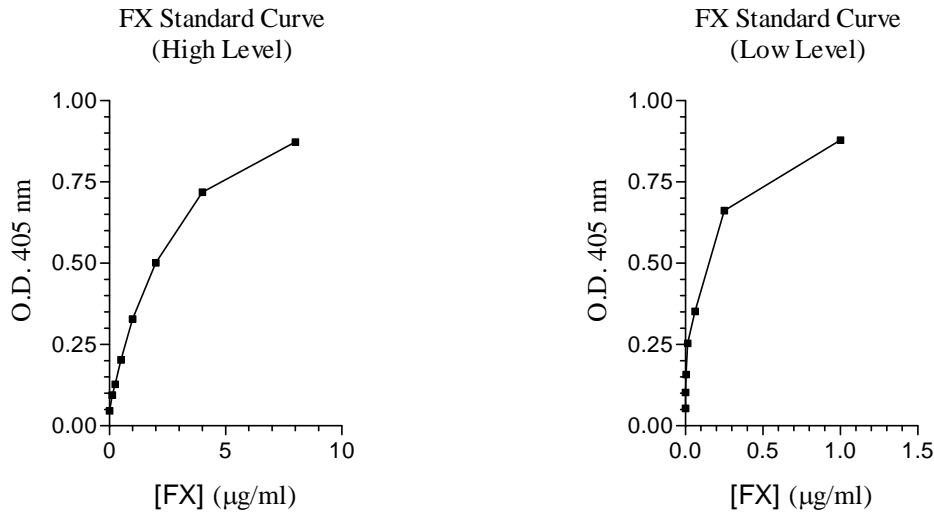
FX or Samples	20 µl
Assay Mix	40 µl
<b>High FX activity Samples:</b> Read the absorbance at 405 nm every 2 minutes at 37°C for 10 minutes.	
<b>Low FX activity Samples:</b> Read the absorbance at 405 nm every 10 minutes at 37°C from 20 to 60 minutes.	

**Data Analysis**

- Calculate the mean value of the duplicate 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 ( $\Delta A/\text{min}$ ) 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 plasma value by the dilution factor of 10.

## Standard Curve



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

## Specificity

- Heparin concentration below 30 U/ml does not interfere with the assay.
- No other enzyme which 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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