Human Factor X
Activity Kit

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Hinweis/Note:
Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

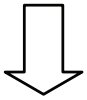
The datasheet is only a first information.
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

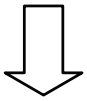
Thank you for choosing Assaypro.

Assay Summary

Add 20 µl of Standard/ Sample and
40 µl of AssayMix per well.
Immediately read absorbance at 405 nm.



Incubate at 37°C.



Read absorbance at 405 nm every
4 minutes for 16 minutes (High FX Activity) or
every 5 minutes for 80 minutes (Low FX Activity).

Assay Template

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								

AssaySense Human Factor X (FX) Chromogenic Activity Kit

Catalog No. CF1010
Sample Insert/Reference Only

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 Factor X Chromogenic Activity Kit is developed to determine human FX activity in plasma, serum, and cell culture samples. 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

- **Prepare all reagents (diluent buffers, substrate, standard, and RVV) as instructed, prior to running the assay.**
- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- 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 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Sample Diluent (6x):** A six-fold concentrate (5 ml).
- **Human FX Standard:** 1 vial, lyophilized (20 μg).
- **Assay Diluent (1x):** A working solution (5 ml).
- **RVV:** 1 vial, lyophilized.
- **FXa Substrate:** 2 vials, lyophilized.

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Opened diluents may be stored for up to 30 days at 2-8°C.
- Store reconstituted standard and reagents at -20°C or below and use within 30 days.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 405 nm
- Pipettes (1-20 μl, 20-200 μl, 200-1000 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. Dilute sample 1:20 with Sample Diluent or within the range of 5x – 40x, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes and remove serum. Dilute sample 1:20 with Sample Diluent or within the range of 5x – 40x, and assay. The undiluted 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. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **Sample Diluent (6x):** If crystals have formed, mix gently until the crystals have completely dissolved. Dilute the Sample Diluent 1:6 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Assay Diluent (1x):** If crystals have formed, mix gently until the crystals have completely dissolved. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the Human FX Standard (20 μg) with 1.25 ml of Sample Diluent to generate a 16 μg/ml standard stock solution. 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 stock solution (16 μg/ml) 1:2 with equal volume of Sample Diluent to produce 8, 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 and used within 30 days.
    - For **low level** of FX Standard, dilute standard stock solution (16 μg/ml) 1:16 with Sample Diluent to produce a 1 μg/ml standard working solution. Prepare duplicate or triplicate standard points by serially diluting the standard working solution 1:4 with 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 and used within 30 days.

Standard curve for high level of FX activity samples:

Standard Point	Dilution	[FX] (μg/ml)
P1	1 part Standard (16 μg/ml) + 1 part Sample Diluent	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 (16 µg/ml) + 15 parts Sample Diluent	1.000
P2	1 part P1 + 3 parts Sample Diluent	0.250
P3	1 part P2 + 3 parts Sample Diluent	0.063
P4	1 part P3 + 3 parts Sample Diluent	0.016
P5	Sample Diluent	0.000

- **RVV:** Add 1.1 ml of Sample Diluent and allow the RVV to sit for 5 minutes to dissolve with gentle agitation prior to using. Any remaining solution should be frozen at -20°C and used within 30 days.
- **FXa Substrate**: Add 1.1 ml of reagent grade water and allow the substrate to sit for 5 minutes to dissolve with gentle agitation prior to using. Any remaining solution should be frozen at -20°C and used within 30 days.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is incubated 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 Human Factor X Standard or sample per well.
- **AssayMix:** At room temperature, freshly prepare the desired volume of the AssayMix by combining the following reagents according to the assay numbers (n) plus one.

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

- Add 40 µl of above AssayMix 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 4 minutes for 16 minutes.
  - For low level of FX activity, read the absorbance every 5 minutes for 80 minutes.

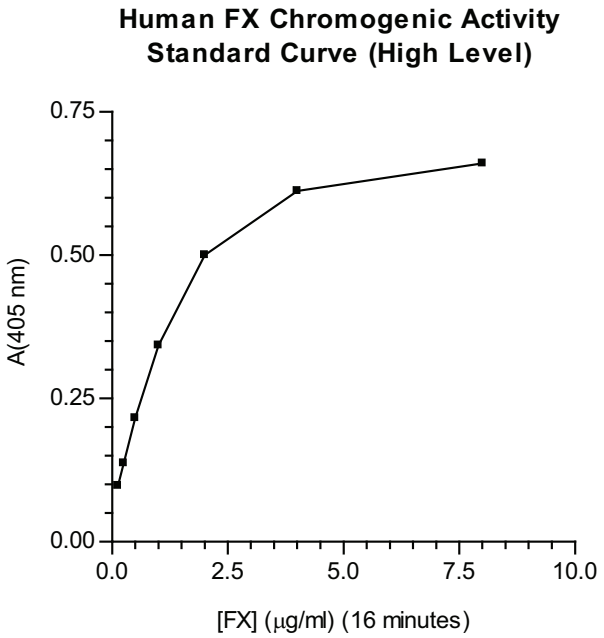
FX Standard or Sample	20 µl
AssayMix	40 µl
Immediately read absorbance at 405 nm.	
High FX activity Samples: Incubate 37°C, read absorbance at 405 nm every 4 minutes for 16 minutes.	
Low FX activity Samples: Incubate 37°C, read absorbance at 405 nm every 5 minutes for 80 minutes.	

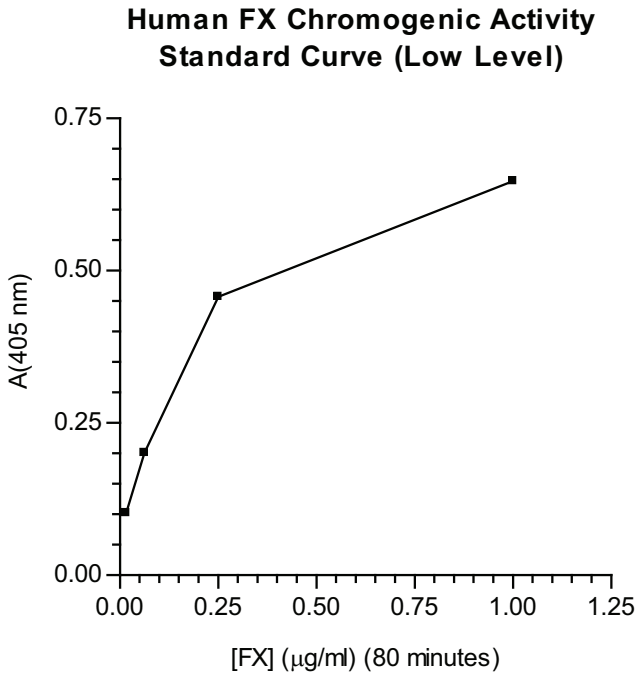
Data Analysis

- 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

- 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.

Version 3.9

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