AssaySense

Human Factor X Chromogenic Activity Kit

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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

Step 1. Add 20 µl of Standard or Sample per well.
Add 40 µl of Assay Mix per well.

Step 2. Read the absorbance at 405 nm for a zero minute background reading.
Cover and incubate at 37°C.

Step 3. Read every 5 minutes for 20 minutes (High Activity).
Read every 5 minutes for 80 minutes (Low Activity).

Symbol Key

Consult instructions for use.

Assay Template

	A	B	C	D	E	F	G	H
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AssaySense Human Factor X Chromogenic Activity Kit

Catalog No. CF1010

Sample insert for reference use 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, as well as 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 the Assay

The AssaySense Human Factor X Chromogenic Activity Kit is developed to determine FX chromogenic activity in human **plasma, serum, urine, and cell culture samples**. The assay measures the activation of zymogen FX to FXa by RVV. The amidolytic activity of 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 product is for **Research Use Only** and is not intended for use in diagnostic procedures.
- Prepare all reagents 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 insert. However, the user should determine the optimal dilution factor.
- The kit should not be used beyond the expiration date.

Reagents

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

- **Microplate:** A 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.
- **Human Factor X Standard:** Lyophilized (9.6 μg).
- **Sample Diluent (6x):** A 6-fold concentrate (5 ml).
- **Assay Diluent (1x):** A working solution (5 ml).
- **RVV:** Lyophilized.
- **FXa Substrate:** 2 vials, lyophilized.

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store Standard, FXa Substrate, and RVV at -20°C.
- Store Microplate, Sample Diluent, and Assay Diluent at 2-8°C.
- Unused microplate wells may be returned to the pouch and resealed.

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 and collect plasma. A 20-fold sample dilution is suggested into Sample Diluent or within the range of 5x – 40x; however, user should determine optimal dilution factor depending on application needs. 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. A 20-fold sample dilution is suggested into Sample Diluent or within the range of 5x – 40x; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes. A 2-fold sample dilution is suggested into Sample Diluent; however, user should determine optimal dilution factor depending on

application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- **Cell Culture Supernatant:** Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatant. If necessary, dilute samples into Sample Diluent; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

Applicable samples may also include biofluids, cell culture, and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater (*for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
A) 4 µl sample: 396 µl buffer (100x) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl.*
1000x	**100000x**
A) 4 µl sample : 396 µl buffer (100x) B) 24 µl of A : 216 µl buffer (10x) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) C) 24 µl of B : 216 µl buffer (10x) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl.*

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **Sample Diluent (6x):** Dilute the Sample Diluent Concentrate 6-fold with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved. 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. After opening, store for up to 30 days at 2-8°C.
- **Human Factor X Standard:** Reconstitute the Human Factor X Standard (9.6 µg) with 0.6 ml of Sample Diluent to generate a 16 µg/ml standard

stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions.

- **High Activity:** Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (16 μg/ml) 2-fold 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 (0 μg/ml).
- **Low Activity:** From the standard stock solution (16 μg/ml), dilute 16-fold 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 μg/ml) 4-fold with Sample Diluent to produce 0.25, 0.063, and 0.016 μg/ml solutions. Sample Diluent serves as the zero standard (0 μg/ml).

Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

Standard Curve for High Activity:

Standard Point	Dilution	[FX] (μg/ml)
P1	1 part Standard (16 μg/ml) + 1 part Sample Diluent	8.0
P2	1 part P1 + 1 part Sample Diluent	4.0
P3	1 part P2 + 1 part Sample Diluent	2.0
P4	1 part P3 + 1 part Sample Diluent	1.0
P5	1 part P4 + 1 part Sample Diluent	0.5
P6	1 part P5 + 1 part Sample Diluent	0.25
P7	1 part P6 + 1 part Sample Diluent	0.125
P8	Sample Diluent	0.0

Standard Curve for Low Activity:

Standard Point	Dilution	[FX] (μg/ml)
P1	1 part Standard (16 μg/ml) + 15 parts Sample Diluent	1.0
P2	1 part P1 + 3 parts Sample Diluent	0.25
P3	1 part P2 + 3 parts Sample Diluent	0.063
P4	1 part P3 + 3 parts Sample Diluent	0.016
P5	Sample Diluent	0.0

- **RVV:** Add 1.1 ml of Sample Diluent to produce a 1x stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to use. Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

- **FXa Substrate:** Add 1.1 ml of reagent grade water to produce a 1x stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to use. Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20°C and **used within 10 days**.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at 37°C in a humid incubator.
- Remove excess microplate strips from the plate frame.
- Freshly prepare the desired volume of the Assay Mix by combining the following reagents according to the number of wells in the assay (n) plus one well.

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

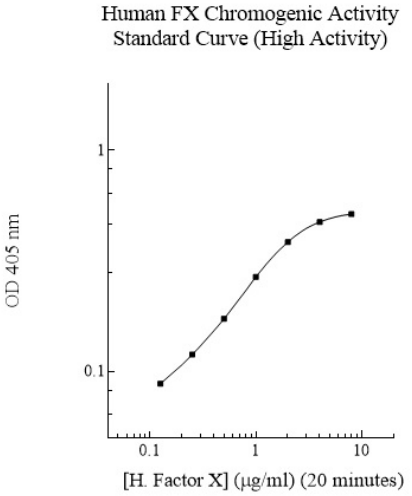
- Add 20 µl of Human Factor X Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Add 40 µl of Assay Mix to each well. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. Read the absorbance at 405 nm for a zero minute background reading. Cover the wells with a sealing tape and incubate at 37°C in a humid incubator to avoid evaporation. Incubate microplate at 37°C after each reading.
- For **high activity**, read the absorbance every 5 minutes for 20 minutes.
- For **low activity**, read the absorbance every 5 minutes for 80 minutes.

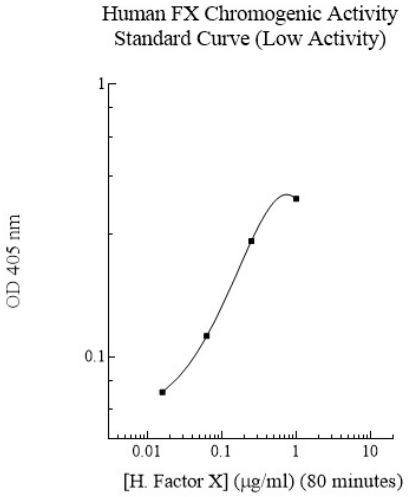
Data Analysis

- Calculate the mean value of the duplicate or triplicate readings 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 after subtracting the background. 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.

Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of improper components	• Check the expiration date listed before use. • Do not interchange components from different lots.
	Splashing of reagents while loading wells	• Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	• Each step of the procedure should be performed uninterrupted.
	Omission of step	• Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	• Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	• Check pipette calibration. • Check pipette for proper performance.
	Improper reagent preparation	• Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	• Consult the provided procedure for correct incubation time.
Deficient Standard Curve Fit	Non-optimal sample dilution	• User should determine the optimal dilution factor for samples.
	Contamination of reagents	• A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	• Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.

References

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(2) Neuenschwander PF *et al.* (1993) *Thrombosis and Haemostasis.* 70:970.
(3) Messier TL *et al*. (1991) *Gene.* 99:291.
(4) Di Scipio RG *et al.* (1977) *Biochemistry.* 16:5253.