



**AssaySense**  
**Human Factor X Chromogenic**  
**Activity Kit**

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Thank you for choosing Assaypro.

## Assay Summary

- Step 1.** Add 20  $\mu\text{l}$  of Standard or Sample per well.  
Add 40  $\mu\text{l}$  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 the absorbance at 405 nm every 5 minutes for 20 minutes (High Activity).  
Read the absorbance at 405 nm every 5 minutes for 80 minutes (Low Activity).  
Cover and incubate at 37°C after each reading.

## Symbol Key



Consult instructions for use.





# AssaySense Human Factor X (Factor 10) 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, and also by the extrinsic (tissue factor/factor VIIa) pathway to give an active FXa enzyme. 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 supernatant 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 96 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:** Calibrated against WHO 4<sup>th</sup> International Standard (7.2 µg, lyophilized).
- **Sample Diluent (6x):** A 6-fold concentrate (5 ml).
- **Assay Diluent (1x):** A working solution (5 ml).
- **RVV:** Lyophilized.
- **FXa Substrate:** Lyophilized, 2 vials.

## 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. (EDTA can also be used as an anticoagulant. Heparin is not recommended.) Centrifuge samples at 3000 x *g* for 10 minutes and collect plasma. A 20-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.
- **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; 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 or within the range of 1x – 10x; 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.**

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

## 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 (7.2 µg, 900 mIU) with 0.9 ml of Sample Diluent to generate an 8 µg/ml

(1000 mIU/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 (8 µg/ml) 2-fold with equal volume of Sample Diluent to produce 4, 2, 1, 0.5, 0.25, 0.125, and 0.063 µg/ml solutions. Sample Diluent serves as the zero standard (0 µg/ml).
- **Low Activity:** From the standard stock solution (8 µg/ml), dilute 8-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)	[FX] (mIU/ml)
P1	1 part Standard + 1 part Sample Diluent	4.0	500
P2	1 part P1 + 1 part Sample Diluent	2.0	250
P3	1 part P2 + 1 part Sample Diluent	1.0	125
P4	1 part P3 + 1 part Sample Diluent	0.5	62.5
P5	1 part P4 + 1 part Sample Diluent	0.25	31.25
P6	1 part P5 + 1 part Sample Diluent	0.125	15.625
P7	1 part P6 + 1 part Sample Diluent	0.063	7.813
P8	Sample Diluent	0.0	0.0

### Standard Curve for Low Activity:

Standard Point	Dilution	[FX] (µg/ml)	[FX] (mIU/ml)
P1	1 part Standard + 7 parts Sample Diluent	1.0	125
P2	1 part P1 + 3 parts Sample Diluent	0.25	31.25
P3	1 part P2 + 3 parts Sample Diluent	0.063	7.813
P4	1 part P3 + 3 parts Sample Diluent	0.016	1.953
P5	Sample Diluent	0.0	0.0

- **RVV:** Add 1.1 ml of Sample Diluent to generate 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 generate a 1x stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to use; keep the vial on ice. 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 to avoid evaporation.
- 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 Reagent	n = 1 well
Assay Diluent	10 $\mu$ l
RVV	10 $\mu$ l
FXa Substrate	20 $\mu$ l

- Add 20  $\mu$ l of Human Factor X Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Add 40  $\mu$ l 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 wells with a sealing tape and incubate at 37°C in a humid incubator. Incubate microplate at 37°C after each reading.
- For **high activity**, read the absorbance at 405 nm every 5 minutes for 20 minutes.
- For **low activity**, read the absorbance at 405 nm 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 optimal 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 after subtracting the background. 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.

## Typical Data

- The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

## High Activity

Standard Point	$\mu\text{g/ml}$	Average OD (20 min)
P1	4.0	0.433
P2	2.0	0.409
P3	1.0	0.346
P4	0.5	0.250
P5	0.25	0.160
P6	0.125	0.110
P7	0.063	0.082
P8	0.0	0.049

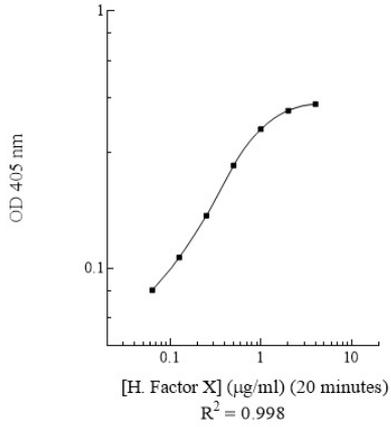
## Low Activity

Standard Point	$\mu\text{g/ml}$	Average OD (50 min)
P1	1.0	0.475
P2	0.25	0.360
P3	0.063	0.175
P4	0.016	0.089
P5	0.0	0.048

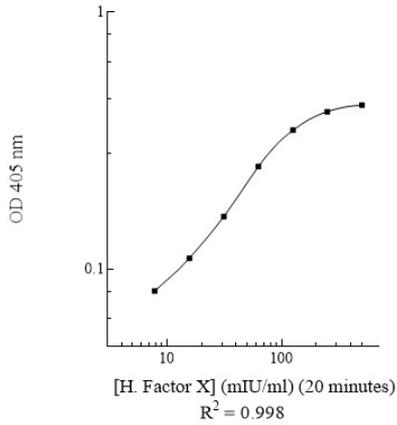
## Standard Curve

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

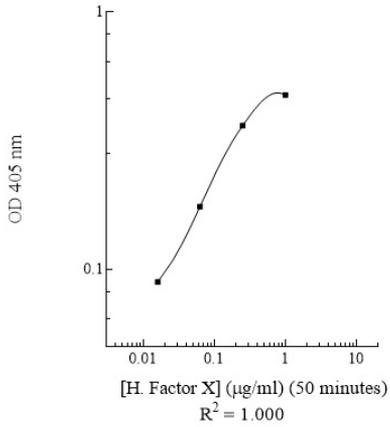
Human FX Chromogenic Activity  
Standard Curve (High Activity)



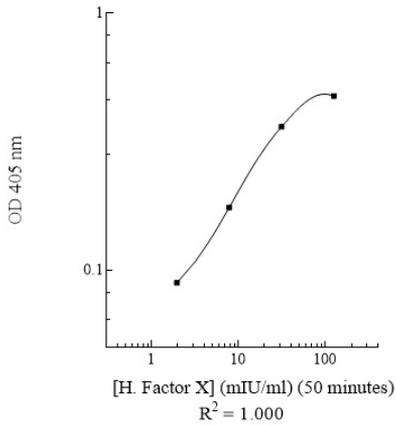
Human FX Chromogenic Activity  
Standard Curve (High Activity)



Human FX Chromogenic Activity  
Standard Curve (Low Activity)



Human FX Chromogenic Activity  
Standard Curve (Low Activity)



## Performance Characteristics

- **Kit standard has been calibrated against WHO International Standard.**
- The minimum detectable dose of human FX at 50 minutes is approximately 9 ng/ml.

## Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of improper components	<ul style="list-style-type: none"> <li>• Check the expiration date listed before use.</li> <li>• Do not interchange components from different lots.</li> </ul>
	Splashing of reagents while loading wells	<ul style="list-style-type: none"> <li>• Pipette properly in a controlled and careful manner.</li> </ul>
	Inconsistent volumes loaded into wells	<ul style="list-style-type: none"> <li>• Pipette properly in a controlled and careful manner.</li> <li>• Check pipette calibration.</li> <li>• Check pipette for proper performance.</li> </ul>
	Insufficient mixing of reagent dilutions	<ul style="list-style-type: none"> <li>• Thoroughly agitate the lyophilized components after reconstitution.</li> <li>• Thoroughly mix dilutions.</li> </ul>
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	<ul style="list-style-type: none"> <li>• Each step of the procedure should be performed uninterrupted.</li> </ul>
	Omission of step	<ul style="list-style-type: none"> <li>• Consult the provided procedure for complete list of steps.</li> </ul>
	Steps performed in incorrect order	<ul style="list-style-type: none"> <li>• Consult the provided procedure for the correct order.</li> </ul>
	Insufficient amount of reagents added to wells	<ul style="list-style-type: none"> <li>• Check pipette calibration.</li> <li>• Check pipette for proper performance.</li> </ul>
	Improper reagent preparation	<ul style="list-style-type: none"> <li>• Consult reagent preparation section for the correct dilutions of all reagents.</li> </ul>
Insufficient or prolonged incubation periods	<ul style="list-style-type: none"> <li>• Consult the provided procedure for correct incubation time.</li> </ul>	
Deficient Standard Curve Fit	Non-optimal sample dilution	<ul style="list-style-type: none"> <li>• User should determine the optimal dilution factor for samples.</li> </ul>
	Contamination of reagents	<ul style="list-style-type: none"> <li>• A new tip must be used for each addition of different samples or reagents during the assay procedure.</li> </ul>
	Contents of wells evaporate	<ul style="list-style-type: none"> <li>• Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.</li> </ul>
	Improper pipetting	<ul style="list-style-type: none"> <li>• Pipette properly in a controlled and careful manner.</li> <li>• Check pipette calibration.</li> <li>• Check pipette for proper performance.</li> </ul>
	Insufficient mixing of reagent dilutions	<ul style="list-style-type: none"> <li>• Thoroughly agitate the lyophilized components after reconstitution.</li> <li>• Thoroughly mix dilutions.</li> </ul>

## References

- (1) Ruf W, Edgington TS. (1994) *FASEB J.* 8:385.
- (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.

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