AssaySense

Human Factor Xa Chromogenic Activity Kit

Assaypro LLC
3400 Harry S Truman Blvd
St. Charles, MO 63301
T (636) 447-9175
F (636) 395-7419
www.assaypro.com

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 100 µl of Standard or Sample per well.
Cover and incubate at 2-8°C overnight or for at least 12 hours.

Step 2. Wash, then add 120 µl of EIA Diluent (1x) and 20 µl of FXa Substrate per well.

Step 3. Read the absorbance at 405 nm for a zero minute background reading.
Cover and incubate at 37°C.

Step 4. Read the absorbance (405 nm) at 22 hours.
Continue reading every hour up to 28 hours.
Cover and incubate at 37°C after each reading.

Symbol Key

Consult instructions for use.

Assay Template

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

AssaySense Human Factor Xa (Activated Factor 10) Chromogenic Activity Kit

Catalog No. CF2010

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 by the 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 Xa Chromogenic Activity Kit is developed to determine FXa activity in human **plasma, serum, and cell culture samples**. The assay couples immunofunctional and direct amidolytic function. A polyclonal antibody specific for human FXa has been pre-coated onto a 96-well microplate with removable strips, and FXa is bound to the immobilized antibody. The amidolytic activity of the FXa is quantitated by 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 FXa 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.
- **Do not prepare FXa substrate until user is ready to continue to the chromogenic activity process.**
- 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.

- **Human Factor Xa Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human FXa.
- **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 Xa Standard:** 1 mIU, lyophilized, 2 vials.
- **FXa Substrate:** Lyophilized, 2 vials.
- **EIA Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (20 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store Standard and FXa Substrate at -20°C.
- Store Microplate, EIA Diluent Concentrate (10x), and Wash Buffer Concentrate (20x) at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.

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. The sample is suggested for use at 1x; 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. The sample is suggested for use at 1x; 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 EIA 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.
- **EIA Diluent Concentrate (10x):** Dilute the EIA Diluent Concentrate 10-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.
- **Human Factor Xa Standard:** Reconstitute the Human Factor Xa Standard (1 mIU, 4.5 ng) with 0.5 ml of EIA Diluent to generate a 2 mIU/ml

(9 ng/ml) standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (2 mIU/ml) 2-fold with equal volume of EIA Diluent to produce 1, 0.5, 0.25, 0.125, 0.063, and 0.031 mIU/ml solutions. EIA Diluent serves as the zero standard (0 mIU/ml). Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

Standard Point	Dilution	[FXa] (mIU/ml)	[FXa] (ng/ml)
P1	1 part Standard	2.0	9.0
P2	1 part P1 + 1 part EIA Diluent	1.0	4.5
P3	1 part P2 + 1 part EIA Diluent	0.5	2.25
P4	1 part P3 + 1 part EIA Diluent	0.25	1.125
P5	1 part P4 + 1 part EIA Diluent	0.125	0.563
P6	1 part P5 + 1 part EIA Diluent	0.063	0.281
P7	1 part P6 + 1 part EIA Diluent	0.031	0.141
P8	EIA Diluent	0.0	0.0

- **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer Concentrate 20-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.
- **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.
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- **The assay is performed at 2-8°C for binding of standard and samples and at 37°C for chromogenic activity.**
- Add 100 µl of Human Factor Xa Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have

formed. Cover wells with a sealing tape and incubate at 2-8°C overnight or for at least 12 hours.

- **Prepare FXa Substrate prior to washing the microplate.** Allow the vial to sit for 10 minutes with gentle agitation prior to use.
- Wash the microplate manually or automatically using a microplate washer. Invert the plate and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If washing manually, wash five times with 200 µl of Wash Buffer per well. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a microplate washer, wash six times with 300 µl of Wash Buffer per well; invert the plate and hit 4-5 times on absorbent material to completely remove the liquid.
- Add 120 µl of EIA Diluent (1x) to each well, and immediately add 20 µl of FXa Substrate 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 to avoid evaporation.
- Read the absorbance (405 nm) at 22 hours and continue reading every hour up to 28 hours. Cover wells with a sealing tape and incubate at 37°C after each reading.

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 (OD) 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 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.

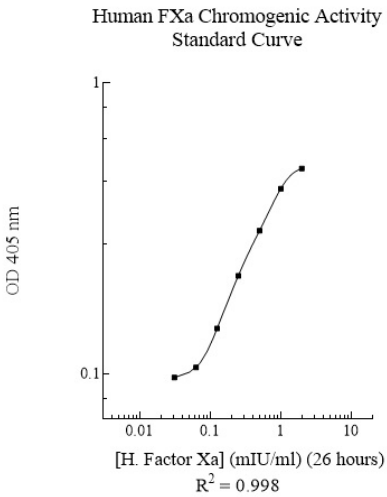
Typical Data

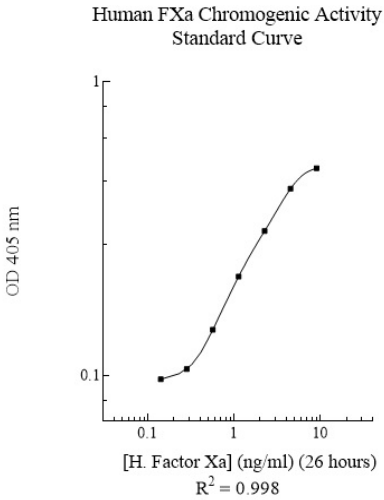
- The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	mIU/ml	Average OD
P1	2.0	0.507
P2	1.0	0.432
P3	0.5	0.310
P4	0.25	0.217
P5	0.125	0.143
P6	0.063	0.105
P7	0.031	0.097
P8	0.0	0.058

Standard Curve

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





Performance Characteristics

- The minimum detectable dose of human FXa is approximately 0.019 mIU/ml.

Notes

- The conversion of mIU and ng is 21.85 mIU/ml = 100 ng/ml.
- The conversion of IU and mIU is 1 IU/ml = 1000 mIU/ml.

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.
	Improper wash step	• Check that the correct wash buffer is being used. • Check that all wells are empty after aspiration. • Check that the microplate washer is dispensing properly. • If washing by pipette, check for proper pipetting technique.
	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.

	Improperly sealed microplate	• Check the microplate pouch for proper sealing. • Check that the microplate pouch has no punctures. • Check that three desiccants are inside the microplate pouch prior to sealing.
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.
	Wash step was skipped	• Consult the provided procedure for all wash steps.
	Improper wash buffer	• Check that the correct wash buffer is being used.
	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

(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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