Human Factor Xa Chromogenic 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

Step 1. Add 100 µl of Standard or Sample per well.
Incubate overnight or for at least 12 hours at 2-8°C.

Step 2. Wash, then add 120 µl of EIA (1x) per well and 20 µl of Factor Xa Substrate per well.
Read at 405 nm at 0 minutes. Incubate at 37°C.

Step 3. Read at 405 nm at 22 hours, and continue reading every 1 hour up to 28 hours.

Symbol Key

Consult instructions for use.

Assay Template

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AssaySense Human Factor Xa (FXa) Chromogenic Activity Kit

Catalog No. CF2010
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 the Assay

The AssaySense Human FXa Chromogenic Activity Kit is developed to determine human FXa activity in **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

- 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 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.

Reagents

- **Human FXa Microplate:** One 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 FXa Standard:** 1 vial, lyophilized (32 mU).
- **Human FXa Substrate:** 2 vials, lyophilized.
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml).
- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (20 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store FXa Standard and FXa Substrate at -20°C.
- Store Microplate, Diluent, Buffers 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.
- Opened Diluents (1x) may be stored for up to 30 days at 2-8°C.

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 assay. 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, remove serum, 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:** Collect cell culture media and centrifuge at 3000 x *g* for 10 minutes at 4°C to remove debris and assay. Samples can be stored at -20°C. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **EIA Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the Human FXa Standard with 4 ml of EIA Diluent to generate an 8 mU/ml standard stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Dilute standard stock solution (8 mU/ml) 1:4 with EIA Diluent to produce a 2 mU/ml standard working solution. Prepare duplicate or triplicate standard points by serially diluting the standard working solution (2 mU/ml) 1:2 with EIA Diluent to produce 1, 0.5, 0.25, 0.125, 0.063, and 0.031 mU/ml solutions. EIA Diluent serves as the zero standard (0 mU/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[FXa] (mU/ml)
P1	1 part Standard + 3 parts EIA Diluent	2.000
P2	1 part P1 + 1 part EIA Diluent	1.000
P3	1 part P2 + 1 part EIA Diluent	0.500
P4	1 part P3 + 1 part EIA Diluent	0.250
P5	1 part P4 + 1 part EIA Diluent	0.125
P6	1 part P5 + 1 part EIA Diluent	0.063
P7	1 part P6 + 1 part EIA Diluent	0.031
P8	EIA Diluent	0.000

- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water. Any remaining solution should be stored at 2-8°C.
- **Human FXa Substrate**: Add 1.1 ml of reagent grade water. 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 performed at 2-8°C for binding of standard and samples and at 37°C for chromogenic activity.**

- **Seal the plate with sealing tape at each step. Be certain that the sealing tape is properly adhered to the plate.**
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely and store in a vacuum desiccator to minimize exposure to water vapor.
- Add 100 µl of Human Factor Xa Standard or sample per well. Cover wells and incubate at 2-8°C overnight or for at least 12 hours.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 120 µl of EIA (1x) and 20 µl of Human Factor Xa Substrate per well. Tap plate to mix gently.

EIA Diluent (1x)	120 µl
FXa Substrate	20 µl

- Read the absorbance (405 nm) at zero minutes for background O.D.
- Seal the plate with sealing tape and incubate at 37°C. Verify that the sealing tape is properly adhered to the plate.
- Read the absorbance (405 nm) at 22 hours and continue reading every 1 hour up to 28 hours.

Standard or Sample	100 ul
Incubate at 2-8°C overnight or for at least 12 hours.	
Wash	
EIA Diluent (1x)	120 µl
FXa Substrate	20 µl
Read the absorbance (405 nm) at zero minutes for background O.D. *Incubate 37°C.* *Verify that the sealing tape is properly adhered to the plate.* *Read the absorbance (405 nm) at 22 hours and continue reading every 1 hour up to 28 hours.*	

Data Analysis

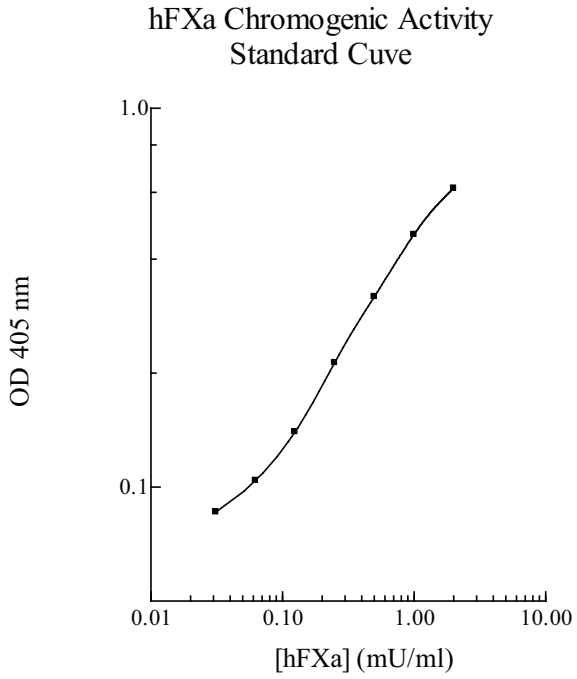
- Calculate the mean value of the duplicate or triplicate for each standard and sample.
- To generate a standard curve from the initial reaction time, plot the graph using the standard concentrations on the x-axis and the corresponding mean 405 nm absorbance or change in absorbance per

minute (ΔA/min) on the y-axis. The best-fit line can be determined by regression analysis 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.

Standard Curve

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



Performance Characteristics

- The minimum detectable dose of FXa is typically ~ 0.01 mU/ml.
- This assay recognizes both natural and recombinant human FXa.

Notes

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

References

(1) Ruf, W. and Edgington, T.S. (1994) *FASEB J*. 8:385
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(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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