Human Factor VIIa 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.

Symbol Key

Consult instructions for use.

Assay Summary

Add 100 µl of Standard/ Sample per well.

⇩

Cover and incubate overnight at room temperature.

⇩

Wash, then add 120 µl of AssayMix and 20 µl of Fx_a Substrate per well.
Read at 405 nm at 0 minutes.

⇩

Cover and incubate at 37°C.

⇩

Read at 20 hours, and continue reading every 1 hour for 26 hours.

Assay Template

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AssaySense Human Factor VIIa (FVIIa) Chromogenic Activity Kit

Catalog No. CF2007
Sample Insert/Reference Only

Introduction

Factor VII (FVII) is a vitamin K-dependent plasma glycoprotein that is synthesized in the liver and circulates in blood as a single-chain inactive zymogen with a molecular mass of 50 kDa (1). Upon tissue damage and vascular injury, the cell surface receptor and cofactor tissue factor (TF) binds and allosterically activates FVII to its active form, FVIIa. The TF/FVIIa complex catalyzes the conversion of both factor IX to factor IXa and factor X to factor Xa to initiate coagulation via the extrinsic pathway (2, 3). Very low levels of FVII are associated with severe coagulation disorders (4). Elevated plasma levels of FVII coagulant activity constitute an independent risk factor for fatal outcomes of coronary heart disease in middle-aged men (5).

Principle of the Assay

The AssaySense Human FVIIa Chromogenic Activity Kit is developed to determine human FVIIa activity in plasma and cell culture samples. The assay couples immunofunctional and indirect amidolytic function. A polyclonal antibody specific for human FVIIa has been pre-coated onto a 96-well microplate with removable strips, and FVIIa is bound to the immobilized antibody. The assay measures the ability of FVIIa to activate factor X (FX) to factor Xa. The amidolytic activity of the FVIIa is quantitated by the amount of FXa produced 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 FVIIa 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

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

- **Human FVIIa Microplate:** One 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human FVIIa.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human FVIIa Standard:** 1 vial, lyophilized (0.5 µg).
- **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).
- **Assay Diluent:** 1x, 20 ml.
- **Human FX:** 2 vials, lyophilized.
- **Human FXa Substrate:** 2 vials, lyophilized.

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store FVIIa Standard, FX, and FXa Substrate at -20°C.
- Store Microplate, Diluent, and Wash Buffer 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. If necessary, dilute samples with **EIA Diluent** within the range of 1:2 to 1:10, 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 FVIIa Standard with 1 ml of **EIA Diluent** to generate a 0.5 µg/ml standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (0.5 µg/ml) 1:4 with **EIA Diluent** to produce 0.125, 0.0313, and 0.0078 µg/ml solutions. EIA Diluent serves as the zero standard (0 µg/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[FVIIa] (µg/ml)	[FVIIa] (AU/ml)
P1	Standard (0.5 µg/ml)	0.5000	28.000
P2	1 part P1 + 3 parts EIA Diluent	0.1250	7.0000
P3	1 part P2 + 3 parts EIA Diluent	0.0313	1.7500
P4	1 part P3 + 3 parts EIA Diluent	0.0078	0.4375
P5	EIA Diluent	0.0000	0.0000

- **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.
- **Assay Diluent (1x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Any remaining solution should be stored at 2-8°C.
- **Human FX:** Add 1.1 ml reagent grade water. Any remaining solution should be frozen at -20°C and used within 30 days.
- **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 room temperature for binding of standard and samples and at 37°C for chromogenic activity assay.**
- **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 Factor VIIa Standard or sample per well. Cover wells and incubate at room temperature 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.
- 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.

Reagents	n=1
Assay Diluent	100 µl
FX	20 µl

- Add 120 µl of the above Assay Mix to each well. Tap to mix gently.
- Add 20 µl of Human FXa Substrate to each well and mix gently. Read the absorbance at 405 nm at zero minutes for background O.D. Seal the plate with sealing tape and incubate at 37°C. Be certain that the sealing tape is properly adhered to the plate.
- Read the absorbance (405 nm) at 20 hours and continue reading every 1 hour through 26 hours.

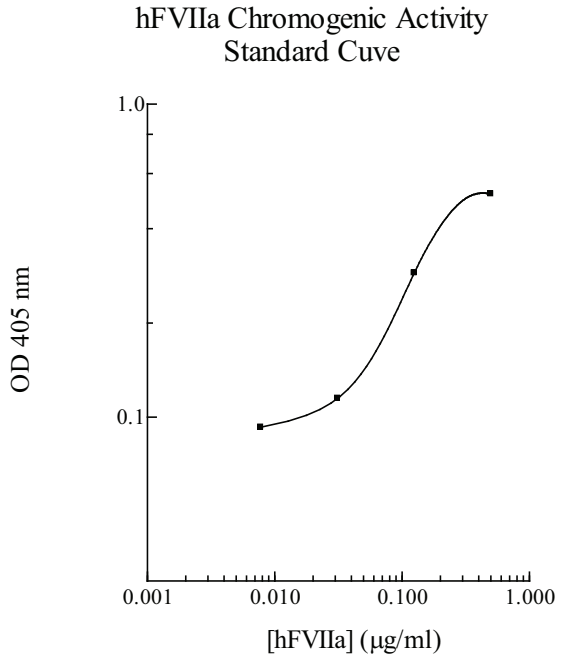
Standard or sample	100 ul
Incubate at room temperature, overnight	
Wash	
Assay Mix	120 µl
FXa Substrate	20 µl
Read the absorbance (405 nm) at zero minutes for background O.D. *Incubate 37°C.* *Be certain that the sealing tape is properly adhered to the plate.* *Read the absorbance (405 nm) at 20 hours and continue reading every 1 hour through 26 hours*	

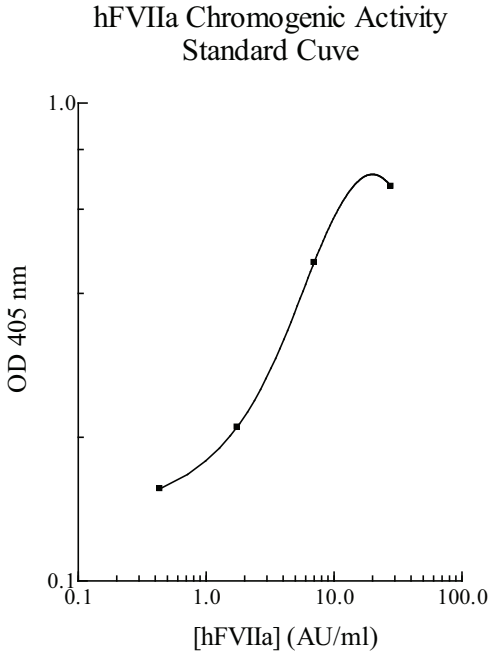
Data Analysis

- 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 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

1. The minimum detectable dose of FVIIa is typically ~ 0.007 µg/ml.
2. This assay recognizes both natural and recombinant human FVIIa.
3. This assay has no FVII activity detected.

References

(1) Davie, E.W. *et al*. (1979) *Adv. Enzyme*. 48:277
(2) Bajaj, S.P. *et al*. (1981) *J. Biol. Chem*. 256:253
(3) Kisiel, W. *et al*. (1975) *Biochemistry* 14:4928
(4) Arbini, A.A. *et al*. (1997) *Blood* 89:176
(5) Junker, R. *et al.* (1997) *Arterioscler. Thromb. Vasc. Biol* 17:1539

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