



AssaySense Human Factor VII (FVII) Chromogenic Activity Assay Kit

Catalog No. CF1007

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 Assay

The AssaySense Human FVII Chromogenic Activity Assay Kit is developed to determine human FVII activity in plasma and cell culture Supernatants. The assay couples immunofunctional and indirect amidolytic assay. A monoclonal antibody specific for human FVII has been pre-coated onto a microplate and active FVII is bound to the immobilized antibody. The assay measures the ability of lipoprotein TF/FVIIa to activate factor X (FX) to factor Xa. The amidolytic activity of the TF/FVIIa complex 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 FVII 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.
- All human source materials have been tested and found to be negative to HbsAg, HIV-1 and HCV by FDA approved methods.

Reagents

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

- **FVII Microplate:** one 96-well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human FVII.

- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human FVII Standard:** 1 vial (3.2 IU)
- **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).
- **Assay Diluent:** 30 ml
- **rhTF (lipoprotein):** 1 vial recombinant human TF lipoprotein.
- **Human FX:** 1 vial
- **FXa Substrate:** 2 vials

Storage Condition

- Store components of the kit at 2-8⁰C or -20⁰C upon arrival up to the expiration date.
- Store FVII Standard, FX, rhTF and FXa Substrate at -20⁰C
- Store Microplate, Diluent Concentrate (10x), and Wash Buffer at 2-8⁰C
- Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.
- Diluent (1x) may be stored for up to 1 month at 2-8⁰C.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 405 nm
- Pipettes (1-20 μ l, 20-200 μ l, and multiple channel)
- Deionized or distilled reagent grade water
- Incubator (37⁰C)

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. Dilute samples 1:8 with EIA Diluent. Store undiluted samples for up to 3 months at -20⁰C or below. Avoid repeated freeze-thaw cycles. (EDTA can also be used as an anticoagulant)
- **Cell Culture Supernatants:** Collect cell culture media and centrifuge at 3000 x g for 10 minutes at 4⁰C to remove debris and assay. Samples can be stored at < -20⁰C. 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 1:10 with reagent grade water. Store for up to 1 month at 2-8⁰C.
- **Standard Curve:** Reconstitute the FVII Standard with 4 ml of EIA Diluent to generate a stock solution of 0.8 IU/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Further dilute the stock solution 1:4 to produce a standard solution of 0.2 IU/ml. Prepare duplicate or triplicate standard points by serially diluting the standard solution (0.2 IU/ml) 1:2 with EIA Diluent to produce 0.1, 0.05, 0.025, and 0.0125 IU/ml. EIA Diluent serves as the zero standard (0 IU/ml). Any remaining solution should be frozen at -20⁰C.

Standard Point	Dilution	[FVII] (IU/ml)
P1	1 part Stock (0.8 IU/ml) + 3 parts EIA Diluent	0.2000
P2	1 part P1 + 1 part EIA Diluent	0.1000
P3	1 part P2 + 1 part EIA Diluent	0.0500
P4	1 part P3 + 1 part EIA Diluent	0.0250
P5	1 part P4 + 1 part EIA Diluent	0.0125
P6	EIA Diluent	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 frozen at -20⁰C.
- **rhTF:** Add 1.2 ml of reagent grade water. Any remaining solution should be frozen at -20⁰C.
- **FX:** Add 1.2 ml reagent grade water. Any remaining solution should be frozen at -20⁰C.
- **Fxa Substrate:** Add 1.1 ml of reagent grade water. Any remaining solution should be frozen at -20⁰C.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature for specific sample binding and at 37⁰C for chromogenic activity assay. Seal the plate with sealing tape at each step.
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely and store in a vacuum desiccator to minimize exposure to water vapor.
- Add 100 µl of standard or sample per well. Cover wells and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel 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	60 µl
rhTF	10 µl
FX	10 µl

- Add 80 µl of the above Assay Mix to each well. Mix gently. Incubate at 37⁰C for 30 minutes.
- Add 20 µl of 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 37⁰C. Read the absorbance at 405 nm every 5 minutes for 20 minutes.

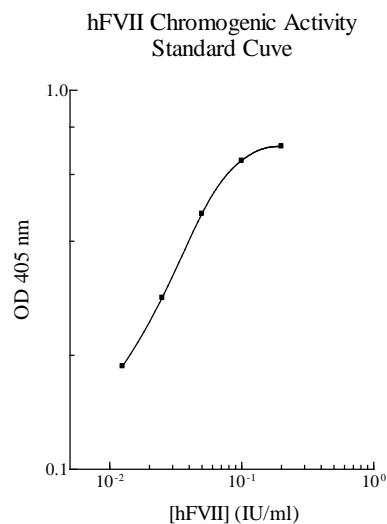
Standard or sample	100 ul
<i>Incubate room temperature, two hours</i>	
<i>Wash</i>	
Assay Mix	80 µl
<i>Incubate 37⁰C, 30 minutes</i>	
FXa Substrate	20 µl
Read the absorbance at 405 nm at zero minutes for background O.D. <i>Incubate 37⁰C, read the absorbance at 405 nm every 5 minutes for 20 minutes</i>	

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 ($\Delta A/\text{min}$) 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 FVII is typically ~ 0.01 IU/ml.
2. This assay recognizes both natural and recombinant human FVII.

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

Version 4.6