AssaySense Human Factor VII (FVII) Chromogenic Activity Assay Kit

Catalog Number CF1007

Introduction

Factor VII (FVII) is a vitamin K-dependent plasma glycoprotein which 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. The assay measures the activation of zymogen FVII to FVIIa by TF lipoprotein. The amidolytic activity of the TF/FVIIa complex is quantitated using a highly specific FVIIa 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

- 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.

- **Microplate:** one 96 well polystyrene microplate (12 strips of 8 wells)

- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Assay Diluent (5x):** 10 ml
- **rhTF (lipoprotein):** 1 vial recombinant human TF lipoprotein
- **Human FVII Standard:** 1 vial
- **FVIIa Substrate:** 1 vial
- **Substrate Dilent:** 5 ml

Storage Condition

- Store unopened kit at 2-8^{0}C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8^{0}C. Store reconstituted standard and reagents at -20^{0}C or below.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 405 nm
- Pipettes (1-20 µl, 20-200 µl, and multiple channel)
- Deionized or distilled reagent grade water
- Incubator (37^{0}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 3,000 x g for 10 minutes and assay. Dilute samples 1:10 with Assay Diluent. Store samples at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- **Cell Culture Supernatants:** Collect cell culture media and centrifuge at 3,000 x g for 10 minutes at 4^{0}C to remove debris. Samples can be store at < -20^{0}C. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- **Standard Curve:** Reconstitute the FVII Standard with 1.2 ml of Assay Diluent to generate a solution of 3 nM. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the standard solution (3 nM) twofold with equal volume of Assay Diluent to produce 1.5, 0.75, 0.375 and 0.188 nM. Assay Diluent serves as the zero standard (0 nM).

Standard Point	Dilution	[FVII] (nM)
P1	1 part Standard	3.000
P2	1 part P1 + 1 part Assay Diluent	1.500
P3	1 part P2 + 1 part Assay Diluent	0.750
P4	1 part P3 + 1 part Assay Diluent	0.375
P5	1 part P4 + 1 part Assay Diluent	0.188
P6	Assay Diluent	0.000

- **Assay Diluent (5x)**: Dilute the Assay Diluent 1:5 with reagent grade water.
- **rhTF:** Add 1.2 ml of Assay Diluent.
- **FVIIa Substrate**: Add 2.2 ml of Substrate Diluent.

Assay Procedure

- To a 96-well plate, add 20 µl of Assay Dilutent to each well.
- Add 10 µl of TF and 10 µl of diluted FVII Standard or testing samples per well. Mix gently.
- Incubate at 37^0C for 30 minutes.
- Add 20 µl of FVIIa Substrate to each well and mix gently. Incubate at 37^0C and read the absorbances at 405 nm every 2 to 5 minutes for 30 minutes (e.g. 2, 5, 10, 15, 20, and 30 min).

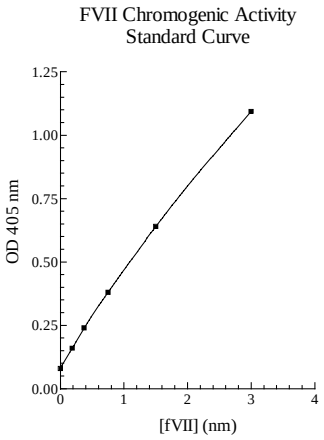
Assay Diluent	20 µl
TF	10 µl
FVII or Samples	10 µl
37^0C, 30 minutes	
FVIIa Substrate	20 µl
37^0C, read the absorbances at 405 nm every 2 to 5 minutes	

Data Analysis

- Calculate the mean value of the triplicate for each standard and sample.
- To generate 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 on the y-axis and draw a best fit curve determined by regression analysis.
- 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

- The minimum detectable dose of FVII is typically < 0.1 nM.
- Intra-assay and inter-assay coefficients of variation were 5.7 % and 4.5% respectively.
- This assay recognizes both natural and recombinant human FVII. No significant cross-reactivity or interference was observed.

References

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