AssaySense

Human Factor VII Chromogenic Activity Kit

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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 2 hours at room temperature.

Step 2. Wash, then add 80 µl of Assay Mix per well.
Incubate 30 minutes at 37°C.

Step 3. Add 20 µl of Factor Xa Substrate per well.
Read the absorbance at 405 nm for a zero minute background reading.
Cover and incubate at 37°C.

Step 4. Read every 5 minutes for 50 minutes at 405 nm.
Cover and incubate at 37°C after each reading.

Symbol Key

Consult instructions for use.

Assay Template

	A	B	C	D	E	F	G	H
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AssaySense Human Factor VII (Factor 7) Chromogenic Activity Kit

Catalog No. CF1007

Sample insert for reference use 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 (FIX) to factor IXa (FIXa) and factor X (FX) to factor Xa (FXa) 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 Factor VII Chromogenic Activity Kit is developed to determine FVII activity in human **plasma, serum, and cell culture samples**. The assay couples immunofunctional and indirect amidolytic function. A polyclonal antibody specific for human FVII has been pre-coated onto a 96-well microplate with removable strips, and active FVII is bound to the immobilized antibody. The assay measures the ability of lipoprotein TF/FVIIa to activate FX to FXa. 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

- 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.
- 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

- **Human Factor VII Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human FVII.
- **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 VII Standard:** Lyophilized (200 mIU, 70 ng).
- **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 (1x):** Buffered protein base (20 ml).
- **rhTF (Lipoprotein):** Recombinant human TF lipoprotein (lyophilized).
- **Human FX:** Lyophilized.
- **FXa Substrate:** Lyophilized, 2 vials.

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store FVII Standard, rhTF, FX, and FXa Substrate at -20°C.
- Store Microplate, EIA Diluent Concentrate (10x), Wash Buffer, and Assay Diluent 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. A 30-fold sample dilution is suggested into EIA Diluent; 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. A 30-fold sample dilution is suggested into EIA Diluent; 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 VII Standard:** Reconstitute the Human Factor VII Standard (200 mIU, 70 ng) with 1 ml of EIA Diluent to generate a 200 mIU/ml (70 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 (200 mIU/ml) 2-fold with equal volume of EIA Diluent to produce 100, 50, 25, and 12.5 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	[FVII] (mIU/ml)
P1	1 part Standard (200 mIU/ml)	200
P2	1 part P1 + 1 part EIA Diluent	100
P3	1 part P2 + 1 part EIA Diluent	50
P4	1 part P3 + 1 part EIA Diluent	25
P5	1 part P4 + 1 part EIA Diluent	12.5
P6	EIA Diluent	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.
- **rhTF:** 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. Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.
- **Human FX:** Add 1.2 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. Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.
- **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 incubated at room temperature for specific sample binding and at 37°C for chromogenic activity steps.**
- Add 100 µl of Human Factor VII 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 for 2 hours at room temperature. Start the timer after the last addition. Prepare Assay Mix Reagent calculations prior to the next step.
- 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.
- 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 well.

Assay Mix Reagent	n = 1 well
Assay Diluent	60 µl
rhTF	10 µl
Human FX	10 µl

- Add 80 µl of Assay Mix 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 for 30 minutes at 37°C in a humid incubator to avoid evaporation.
- 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.
- Read the absorbance at 405 nm every 5 minutes for 50 minutes. Cover and incubate microplate 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 of the 4-parameter curve.

- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

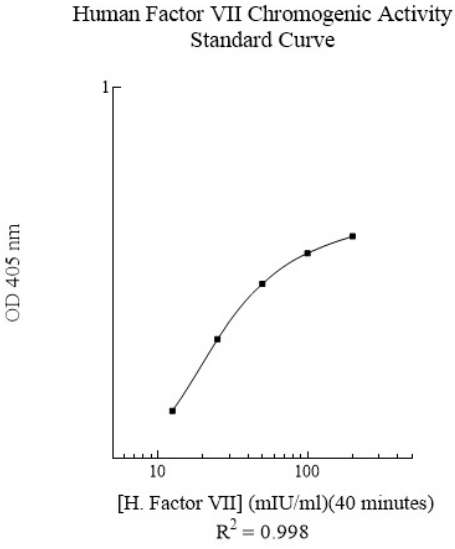
Typical Data

- 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	200	0.454
P2	100	0.415
P3	50	0.353
P4	25	0.263
P5	12.5	0.180
P6	0.0	0.055

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 FVII at 40 minutes as calculated by 2SD from the mean of a zero standard was established to be 4 mIU/ml.
- Intra-assay precision was determined by testing three plasma samples twenty times in one assay.
- Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	5.1%	4.7%	4.6%	9.3%	9.8%	10.9%
Average CV (%)	4.8%			10.0%		

Linearity

- Plasma and serum samples were serially diluted to test for linearity.

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
15x	96%	94%
30x	101%	98%
60x	104%	107%

Notes

- The conversion of mIU to ng is 1 mIU/ml = 0.35 ng/ml.
- The conversion of IU to 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

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(5) Junker R *et al*. (1997) *Arterioscler Thromb Vasc Biol*. 17:1539.

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