ab137995 – Factor VIIa Human Chromogenic Activity Assay Kit

Instructions for Use

For the quantitative measurement of Human Factor VIIa in cell culture supernatants, serum and plasma.

This product is for research use only and is not intended for diagnostic use.

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1. 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. 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. Very low levels of FVII are associated with severe coagulation disorder. Elevated plasma levels of FVII coagulant activity constitute an independent risk factor for fatal outcomes of coronary heart disease in middle-aged men.

Abcam's Factor VIIa Human Chromogenic Activity Assay Kit (ab137995) is developed to determine Human Factor VIIa activity in plasma, serum and cell culture supernatant samples. This kit is also validated for use with bovine, equine, monkey, mouse, rat, swine, and rabbit samples. The assay couples immunofunctional and indirect amidolytic function. A polyclonal antibody specific for Human Factor VIIa has been pre-coated onto a 96-well microplate with removable strips and Factor VIIa is bound to the immobilized antibody. The assay measures the ability of Factor VIIa to activate factor X (FX) to factor Xa. The amidolytic activity of the Factor VIIa 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 Factor VIIa enzymatic activity.

2. Assay Summary

Prepare all reagents, samples and standards as instructed.

Add 100 µl standard or sample to each well.

Incubate at 2-8°C overnight or for at least 12 hours.

Wash the microplate 5 x with wash buffer.

Add 120 µl Assay Mix to each well.

Add 20 µl of Factor Xa Substrate to each well.

Read at 405 nm immediately for background O.D.

Incubate at 37°C, read the absorbance at 405 nm

at 20 hours and continue for every 1 hour up to

28 hours. Cover and incubate between each reading.

3. Storage and Handling

- Store kit at 4°C immediately upon receipt, apart from the Human Factor VIIa Standard, Human Factor X, and Factor Xa Substrate, which should be stored at -20°C.
- 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.
- Opened Assay and 10x Diluent M may be stored for up to 1 month at 2-8°C.

4. Materials supplied

Item	Amount	Storage Condition (Before Preparation)
Human Factor VIIa Microplate (12 x 8 well strips)	96 wells	4°C
Human Factor VIIa Standard	1 vial	-20°C
10X Diluent M Concentrate	20 mL	4°C
Assay Diluent	20 mL	4°C
Human Factor X	2 vial	-20°C
Factor Xa Substrate	2 vials	-20°C
20X Wash Buffer Concentrate	30 mL	4°C
Sealing Tapes	3	N/A

5. Additional Materials Required

- Microplate reader capable of measuring absorbance at 405nm.
- Precision pipettes to deliver 1 µl to 1,000 µl volumes.
- Distilled or deionized reagent grade water.
- Incubator (37°C).

6. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not prepare Factor X protein and FXa substrate until user is ready to continue to the chromogenic activity process.
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

7. Technical Hints

- It is recommended that all standards, controls and samples be run in duplicate. Standards and samples must be assayed at the same time.
- The coefficient of determination of the standard curve should be ≥ 0.95 and the highest O.D. should be more than 1.0.
- Cover or cap all kit components and store at 2-8° C when not in use.
- Microtiter plates should be allowed to come to room temperature before opening the foil bags. Once the desired number of strips has been removed, immediately reseal the bag with desiccants and store at 2-8°C to maintain plate integrity.
- The assay is incubated 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.
- Samples should be collected in pyrogen/endotoxin-free tubes.
- When possible, avoid use of badly hemolyzed or lipemic serum. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.

8. Sample Collection

- **Plasma**

  Collect plasma using a final concentration of 0.1 M sodium citrate as an anticoagulant (EDTA or Heparin can also be used as an anticoagulant). Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1X Diluent M and assay. 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 and remove serum. Dilute samples 1X Diluent M and assay. 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.

9. Preparation of Reagents

Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

1. **1X Diluent M:** Dilute the 10x Diluent M Concentrate (1:10 with reagent grade water). Store for up to 30 days at 2-8°C.
2. **Assay Diluent (1x):** Store for up to 1 month at 2 to 8°C.
3. **Standard Preparations:**

- Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.
- Any remaining standard should be stored at -20°C after reconstitution and used within 48 hours.
- This procedure prepares sufficient standard dilutions for duplicate wells.

- Reconstitute of the Factor VIIa Standard vial to prepare the 8000 ng/mL Factor VIIa **Stock Standard**.
  - First consult the Factor VIIa Standard vial to determine the mass of protein in the vial.
  - Calculate the appropriate volume of 1X Diluent M to add when resuspending the Factor VIIa Standard vial to produce a 8000 ng/mL Factor VIIa **Stock Standard** by using the following equation:

C_S = Starting mass of Factor VIIa Standard (see vial label) (ng)

C_F = 8000 ng/mL Factor VIIa **Stock Standard** final required concentration

V_D = Required volume of 1X Diluent M for reconstitution (µL)

Calculate total required volume 1X Diluent M for resuspension:

$$(C_S / C_F) \times 1{,}000 = V_D$$

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

C_S = 9600 ng of Factor VIIa Standard in vial

C_F = 8000 ng/mL Factor VIIa **Stock Standard** final concentration

V_D = Required volume of 1X Diluent M for reconstitution

$$(9600 \text{ ng} / 8000 \text{ ng/mL}) \times 1{,}000 = 1{,}200 \text{ µL}$$

- First briefly spin the Factor VIIa Standard Vial to collect the contents on the bottom of the tube.
- Reconstitute the Factor VIIa Standard vial by adding the appropriate calculated amount VD of 1X Diluent M to the vial to generate the 8000 ng/mL Factor VIIa Stock Standard. Mix gently and thoroughly.

- Allow the reconstituted 8000 ng/mL Factor VIIa **Stock Standard** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions
- Label six tubes #1 – 6.
- Prepare the 2000 ng/mL **Standard #1** by diluting 75 µL of Factor VIIa Stock Standard (8000 ng/mL) 4-fold with 225 µL Diluent M. Mix thoroughly and gently.
- Add 225 µL of 1X Diluent M to tubes #2 – 7.
- To prepare **Standard #2**, add 75 µL of the **Standard #1** into tube #2 and mix gently.
- To prepare **Standard #3**, add 75 µL of the **Standard #2** into tube #3 and mix gently.
- Using the table below as a guide, prepare subsequent serial dilutions.
- 1X Diluent M serves as the zero standard, 0 ng/mL (tube #6)

Remaining stock solution should be stored at -20°C and used within 30 days. Avoid freeze-thaw cycles.

Standard Dilution Preparation Table

Standard #	Volume to Dilute (µL)	Volume Diluent M (µL)	Total Volume (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	75 µL of 8000 ng/mL Stock Standard to 225 µL Diluent M				2000
2	75	225	300	2000	500
3	75	225	300	500	125
4	75	225	300	125	31.35
5	75	225	300	31.35	7.813
6	-	225	300	-	0.0

4. **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer Concentrate 1:20 with reagent grade water. Any remaining solution should be stored at 2-8°C.
5. **Human Factor X:** Add 1.3 mL reagent grade water. Allow the vial to sit for 10 minutes with gentle agitation prior to use; keep the vial on ice. Any remaining solution should be frozen at -20°C and used within 30 days.
6. **Human Factor Xa Substrate:** Add 1.1 mL of reagent grade water. Allow the vial to sit for 10 minutes with gentle agitation prior to use; keep the vial on ice. Any remaining solution should be frozen at -20°C and used within 30 days.

10. Assay Method

1. Prepare all reagents, standards solutions and samples for initial setup as instructed. Bring all reagents to room temperature before use.
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3. **The assay is incubated at 2-8°C for binding of standard and samples and at 37°C for chromogenic activity steps**. Cover the plate with sealing tape after each step ensuring that the sealing tape is properly adhered to the plate.

4. Add 100 µl of standard or sample to each well. Gently tap plate to thoroughly coat the wells. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate overnight at 2-8°C or for at least 12 hours. Prepare Assay mix reagent calculations prior to the next step.
5. **Prepare FX protein and FXa substrate prior to washing the microplate**. Allow the vials to sit for 10 minutes with gentle agitation prior to use.

6. Wash five times with 200 µl of Wash Buffer manually. Invert the plate and decant the contents, and blot it on absorbent paper towel to complete remove liquid at each step.

7. 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
Human Factor X	20 µL

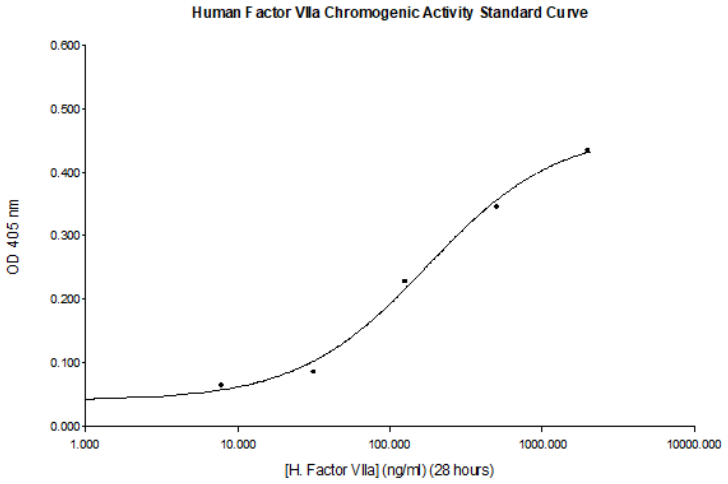
8. Add 120 µL of the above Assay Mix to each well and **immediately** add 20 µL of Human Factor Xa Substrate to each well. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. Read the absorbance at 405 nm for a minute background reading. Cover the plate with sealing tape and incubate at 37°C. Read the absorbance at 405 nm at 20 hours and continue reading every 1 hour through 28 hours. Incubate microplate at 37°C after each reading.

11. Data Analysis

Calculate the mean value of the duplicate or triplicate readings 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 after subtracting the background. 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.

A. Typical Data

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



B. Sensitivity

The minimum detectable dose of Factor VIIa is typically 6.9 ng/mL.

12. Troubleshooting

Problem	Cause	Solution
Poor standard curve	Improper standard dilution	Confirm dilutions made correctly
	Standard improperly reconstituted (if applicable)	Briefly spin vial before opening; thoroughly resuspend powder (if applicable)
	Standard degraded	Store sample as recommended
	Curve doesn't fit scale	Try plotting using different scale
Low signal	Target present below detection limits of assay	Decrease dilution factor; concentrate samples
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding STOP solution	Read plate immediately after adding STOP solution

Problem	Cause	Solution
Low sensitivity	Improper storage of Assay kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

Technical Support

For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)