ab108830

Factor VII Activity Assay Kit (Human, Colorimetric)

Instructions for Use

For the quantitative measurement of Human Factor VII concentrations in plasma, serum and cell culture supernatants

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 disorders. Elevated plasma levels of FVII coagulant activity constitute an independent risk factor for fatal outcomes of coronary heart disease in middle-aged men.

ab108830 Factor VII Activity Assay Kit (Human, Colorimetric) is developed to determine Human FVII activity in plasma, serum and cell culture supernatants. The assay couples immunofunctional and indirect amidolytic assay. A polyclonal 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 (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.

2. Assay Summary

Prepare all reagents, samples and standards as instructed.

Add 100 µl standard or sample to each well.

Incubate 2 hours at room temperature.

Wash the microplate 5 x with wash buffer.

Prepare Assay mix and add 80 µl to each well.

Incubate 30 minutes at 37°C.

Add 20 µl of FXa Substrate to each well.

Read at 405 nm immediately.

Cover and incubate at 37°C.

Incubate at 37°C and read at 405 nm

every 5 minutes for 50 minutes.

3. Kit Contents

- Human FVII 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 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- Human FVII Standard: 1 vial (lyophilized)
- 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: 20 ml
- rhTF (lipoprotein): 1 vial recombinant Human TF lipoprotein.
- Human FX: 1 vial.
- FXa Substrate: 2 vials.

4. Storage and Handling

Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date.
Store FVII Standard, FX, rhTF and FXa Substrate at -20°C.
Store Microplate, Assay Diluent, 10X Diluent M, and 20X Wash Buffer at 2-8°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. Diluent (1x) may be stored for up to 1 month at 2-8°C.

5. Additional Materials Required

- Microplate reader capable of measuring absorbance at 405nm.
- Precision pipettes to deliver 1 µl to 1 ml volumes.
- Distilled or deionized reagent grade water.
- Incubator 37^{o}C

6. Limitations

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- 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.
- 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.
- 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.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
- Do not mix or interchange different reagent lots from various kit lots.
- Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Solution provided. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.

8. Preparation of Reagents

Sample Collection:

1. **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. A 30-fold sample dilution is suggested into Diluent; however, user should determine optimal dilution factor depending on application needs. Store undiluted samples at -20°C or below, for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA can also be used as an anticoagulant
2. **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 Diluent; however, user should determine optimal dilution factor depending on application needs. Store undiluted samples for up to 3 months at -20°C or below. Avoid repeated freeze-thaw cycles.
3. **Cell culture supernatants:** Collect cell culture media and centrifuge at 1500 rpm for 10 minutes at 4°C to remove debris. If necessary, dilute samples into Diluent M; user should determine optimal dilution factor depending on application needs. The undiluted samples Samples can be store at -80°C. Avoid repeated freeze-thaw cycles.

Reagent Preparation:

1. 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.
2. **Diluent Concentrate (10x):** Dilute the Diluent 1:10 with reagent grade water. Store for up to 1 month at 2 to 8°C.
3. **Standard Curve:** Reconstitute the Human FVII Standard with the appropriate amount of Diluent to generate a stock solution of 0.2

IU/ml. 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 (0.2 IU/ml) 1:2 with Diluent to produce 0.1, 0.05, 0.025 and 0.0125 IU/ml. Diluent serves as the zero standard (0 IU/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[FVII] (IU/ml)
P1	1 part Stock (0.2 IU/ml)	0.2000
P2	1 part P1 + 1 part Diluent	0.1000
P3	1 part P2 + 1 part Diluent	0.0500
P4	1 part P3 + 1 part Diluent	0.0250
P5	1 part P4 + 1 part Diluent	0.0125
P6	Diluent	0.0000

4. **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
5. **rhTF:** Add 1.1 ml of reagent grade water. Any remaining solution should be frozen at -20°C and used within 30 days.
6. **Human FX:** Add 1.2 ml of reagent grade water. Any remaining solution should be frozen at -20°C and used within 30 days.
7. **FXa Substrate**: Add 1.1 ml of reagent grade water. Any remaining solution should be frozen at -20°C and used within 30 days.

9. Assay Method

1. 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 37°C for chromogenic activity assay. Seal the plate with sealing tape at each step.
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Seal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3. Add 100 µl of Human Factor VII standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
4. Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; tap 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; tap it 4-5 times on absorbent paper towel to completely remove the liquid.
5. 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

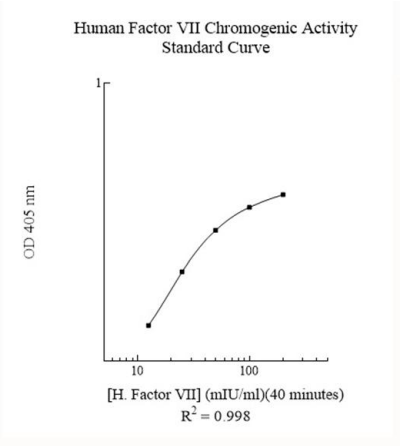
6. Add 80 µl of the above Assay Mix to each well. Mix gently. Incubate at 37°C for 30 minutes.
7. Add 20 µl of FXa Substrate to each well and mix gently. Read the absorbance at 405 nm at zero minutes for background O.D. Seal the plate with sealing tape and incubate at 37°C. Read the absorbance at 405 nm every 5 minutes for 50 minutes. Incubate microplate after each reading.

10. 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 of the linear portion of the curve. 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 FVII is typically 4 mIU/ml. This assay recognizes both natural and recombinant Human FVII.

C. Linearity of Dilution

Plasma Dilution	Average % Expected Value
1:15	96
1:30	101
1:60	104

Serum Dilution	Average % Expected Value
1:15	94
1:30	98
1:60	107

D. Precision

	Intra-Assay	Inter-Assay
% CV	4.8	10.0%

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.*

11. 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	Incubation time too short	Try overnight incubation at 4°C
	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

Problem	Cause	Solution
High background	Wells are insufficiently washed Contaminated wash buffer	Wash wells as per protocol recommendations Make fresh wash buffer
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes and ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (eg. minimize freeze/thaws cycles)
Low sensitivity	Improper storage of ELISA 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:
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