

INTENDED USE

This human coagulation Factor VII antigen assay is intended for the quantitative determination of total Factor VII antigen in human plasma.

For research use only.

BACKGROUND

Factor VII is a single chain glycoprotein zymogen that initiates the extrinsic coagulation pathway by forming a complex with exposed Tissue Factor and Ca^{2+} [1]. Factor VII in complex is rapidly cleaved to VIIa which activates Factor X and IX [2]. Congenital Factor VII deficiency is rare and caused by heterogeneous decreased activity or antigen levels [3]. Elevated Factor VII is associated with increased risk of coronary heart disease [4].

ASSAY PRINCIPLE

Human Factor VII will bind to the capture antibody coated on the microtiter plate. Factor VII, VIIa, and VII in complex with Tissue Factor will react with the antibody on the plate. After appropriate washing steps, monoclonal anti-human Factor VII primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody, which is proportional to the total Factor VII present in the samples, is reacted with the secondary antibody. Following an additional washing step, TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of human Factor VII. Color development is proportional to the concentration of Factor VII in the samples.

STANDARD CALIBRATION

Factor VII standard provided is calibrated against the WHO 4th International Standard for Blood Coagulation Factors II, VII, IX, X, Plasma, Human distributed by NIBSC (09/172), South Mimms, Potters Bar, Hertfordshire, UK.

Lot 216L-A: 120 ng = 0.355 IU

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-human factor VII antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Human Factor VII standard:** 1 vial lyophilized standard
- **Anti-human Factor VII primary antibody:** 1 vial lyophilized monoclonal antibody
- **Anti-mouse horseradish peroxidase secondary antibody:** 1 vial concentrated HRP labeled antibody
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- TBS buffer: 0.1M Tris, 0.15M NaCl, pH 7.4
- Blocking buffer (BB): 3% BSA (w/v) in TBS
- 1X Wash buffer: Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water

SAMPLE COLLECTION

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard

Reconstitute standard by adding 1.2ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 100ng/ml standard solution.

Dilution table for preparation of human Factor VII standard:

Factor VII concentration (ng/ml)	Dilutions
100	500 μl (from vial)
50	500 μl (BB) + 500 μl (100ng/ml)
20	600 μl (BB) + 400 μl (50ng/ml)
10	500 μl (BB) + 500 μl (20ng/ml)
5	500 μl (BB) + 500 μl (10ng/ml)
2	600 μl (BB) + 400 μl (5ng/ml)
1	500 μl (BB) + 500 μl (2ng/ml)
0.5	500 μl (BB) + 500 μl (1ng/ml)
0.2	600 μl (BB) + 400 μl (0.5ng/ml)
0	500 μl (BB) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition

Remove microtiter plate from bag and add 100 μl Factor VII standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 μl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures Factor VII antigen in the 0.2-100 ng/ml range. If the unknown is thought to have high Factor VII levels, dilutions may be made in blocking buffer before use. A 1:20-1:100 dilution for normal plasma is suggested for best results.

Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer to the vial and agitate gently to mix. Add 100 μl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 μl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Secondary Antibody Addition

Briefly centrifuge vial before opening. Dilute 1 μl of conjugated secondary antibody in 10ml of blocking buffer and add 100 μl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 μl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H₂SO₄ or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

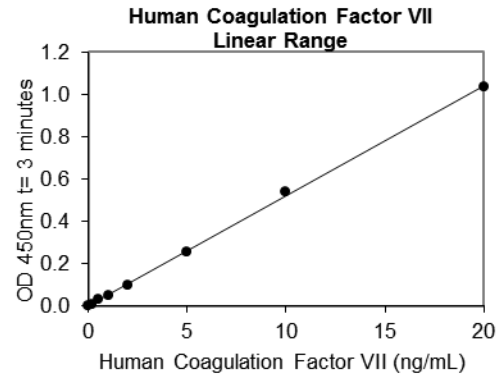
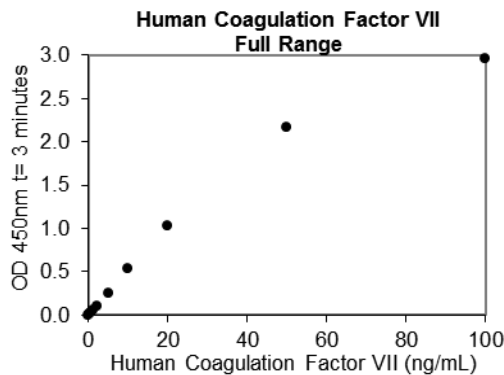
Measurement

Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A₄₅₀).

Calculation of Results

Plot A₄₅₀ against the amount of Factor VII in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of Factor VII in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):



EXPECTED VALUES

The concentration of Factor VII in normal human plasma (n=41) is 470±112 ng/ml [5].

PERFORMANCE CHARACTERISTICS

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD₄₅₀: 0.066 -0.081) and calculating the corresponding concentration. The MDD was 0.164ng/ml.

Intra-assay Precision: These studies are currently in progress. Please contact us for more information.

Inter-assay Precision: These studies are currently in progress. Please contact us for more information.

Recovery: These studies are currently in progress. Please contact us for more information.

Linearity: These studies are currently in progress. Please contact us for more information.

Specificity: These studies are currently in progress. Please contact us for more information.

Sample Values: Samples were evaluated for the presence of the antigen at varying dilutions.

Sample Type	Dilution	Mean (ng/mL)
EDTA Plasma	1:20	421.3
	1:40	440.2
	1:80	403.7

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide.

The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

REFERENCES

1. McVey JH: Baillieres Best Pract Res Clin Haematol. 1999, 12(3):361-72.
2. Bajaj SP, *et al.*: J Biol Chem. 1981, 256(1):253-9.
3. Mariani G, *et al.*: Thromb Haemost. 2005, 93(3):481-7.
4. Junker R, *et al.*: Arterioscler Thromb Vasc Biol. 1997, 17(8):1539-44.
5. Fair DS: Blood. 1983, 62(4):784-91.

Example of ELISA Plate Layout

96 Well Plate: 20 Standard wells, 76 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml		
B	0	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml		
C												
D												
E												
F												
G												
H												

SAMPLE INSERT
 Refer to kit box for
 lot specific instructions