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Human Factor VII Total Antigen Assay ELISA Kit

Strip well format. Reagents for up to 96 tests

Catalog No.	**CS220A**	**Quantity:**	**1 x 96 tests**
	CS220B		**5 x 96 tests**

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

Background: Factor VII is a single chain glycoprotein zymogen that initiates the extrinsic coagulation pathway by forming a complex with exposed Tissue Factor and Ca2+. Factor VII in complex is rapidly cleaved to VIIa which activates Factor X and IX. Congenital Factor VII deficiency is rare and caused by heterogeneous decreased activity or antigen levels. Elevated Factor VII is associated with increased risk of coronary heart disease.

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, polyclonal 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 Calib.: 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 211L: 100 ng = 0.825 IU

Reagents Provided:
♦96-well microtiter strip plate:
8x12 removable well strips containing anti-human Factor VII capture antibody dried and blocked on the surface
♦10X Wash Buffer:
1 bottle of 50ml; bring to 1x using DI water
♦Human Factor VII standard:
1 vial of lyophilized standard
♦Anti-human Factor VII primary antibody:
1 vial of lyophilized polyclonal antibody
♦Anti-rabbit secondary antibody:
1 vial concentrated HRP labeled antibody
♦TMB substrate solution:
1 bottle of 10ml solution

All kit components must be stored at 4°C. Store unopened plate and any unused microtiter

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Storage and Stability: strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -70°C for later use. DO NOT freeze/thaw the standards and primary antibody more than once. All other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.

Reagents and Equipment Required:
- 1-channel pipettes covering 0-10µl and 200-1000µl
- 12-channel pipette covering 30-300µl
- Paper towels or kimwipes
- 50ml tubes, 1.5 ml centrifuge tubes
- 1N H_2SO_4
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- Microtiter plate spectrophotometer operable at 450nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300rpm.

Warnings: Warning – Avoid skin and eye contact when using TMB One substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

Precautions:
- **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.
- **DO NOT** pipette reagents by mouth.
- Always pour TMB substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the TMB substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **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 in TBS
- Wash buffer concentrate: The wash buffer supplied in a 10X concentrate and must be diluted 1:10 with deionized water for use with the kit.

Specimen Collection: The assay measures total human Factor VII in the 0.1-20 ng/ml range. Samples giving human Factor VII levels above 20ng/ml should be diluted in blocking buffer before use. A 1:10 dilution for plasma is suggested for best results.

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

Preparation of Standard:
Reconstitute standard as directed on vial to give a 100 ng/ml standard solution.

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Dilution table for preparation of human Factor VII standards:

Factor VII concentration (ng/ml)	Dilutions
20	800µl (BB) + 200µl (100ng/ml)
10	250µl (BB) + 250µl (20ng/ml)
5	250µl (BB) + 250µl (10ng/ml)
2	300µl (BB) + 200µl (5ng/ml)
1	250µl (BB) + 250µl (2ng/ml)
0.5	250µl (BB) + 250µl (1ng/ml)
0.2	300µl (BB) + 200µl (0.5ng/ml)
0.1	250µl (BB) + 250µl (0.2ng/ml)
0	500µl (BB) Zero point to determine background

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

Standard and Unknown Addition:
Remove microtiter plate from bag. Add 100 µL 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.

Primary Antibody Addition:
Add 10 ml of blocking buffer directly to the primary antibody vial and agitate gently to completely dissolve contents. Add 100 µl to all wells. Shake plate at 300 rpm 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:
Dilute 1 µL into 10 mL 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 100 µl 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 50 µl of 1N H_2SO_4 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 and read final absorbance values at 450 nm. For best results read plate immediately.

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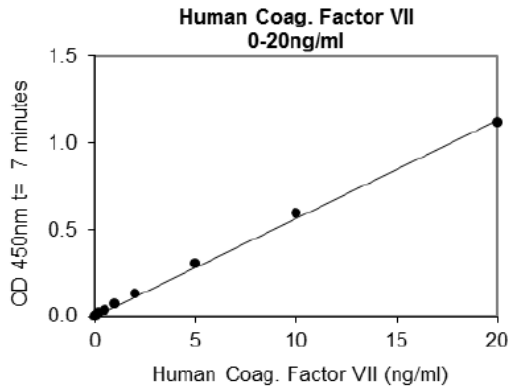
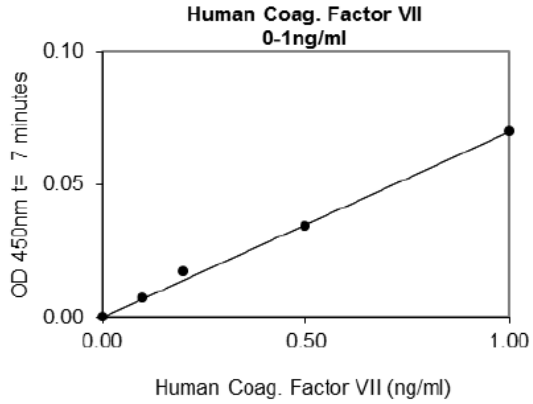
Measurement:
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Assay Calibration:
Plot A_{450} against the amount of human Factor VII in the standards. Fit a straight line through the points using a linear fit procedure. The amount of total human Factor VII in the unknowns can be determined from this curve..

A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)





Expected Values: The concentration of Factor VII in normal human plasma (n=41) is 470±112 ng/ml.

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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 or from contact with the above product.

Example of ELISA Plate Layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.1ng/ml	0.2ng/ml	0.5ng/ml	1.0ng/ml	2.0ng/ml	5.0ng/ml	10ng/ml	20ng/ml			
B	0	0.1ng/ml	0.2ng/ml	0.5ng/ml	1.0ng/ml	2.0ng/ml	5.0ng/ml	10ng/ml	20ng/ml			
C												
D												
E												
F												
G												
H												

96 Well Plate
Standards: 18 Wells
Samples: 78 Wells

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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