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Human Coagulation Factor V ELISA Kit

Strip well format. Reagents for up to 96 tests

Catalog No.	**CS364A** **CS364B**	**Quantity:**	**1 x 96 tests** **5 x 96 tests**

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

Background: Factor V (aka proaccelerin or labile factor) is a 2224 amino acid single chain glycoprotein. Factor V is activated to Factor Va by thrombin. Factor Va binds to Factor Xa and acts as a cofactor in accelerating the activation of prothrombin to thrombin. A genetic Factor V R506Q mutation has been shown to result in a resistance to activated protein C leading to venous thrombosis.

Assay Principle: Human Factor V will bind to the capture monoclonal antibody coated on the microtiter plate. Factor V and Va will react with the antibody on the plate. After appropriate washing steps, biotinylated primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody is reacted with horseradish peroxidase conjugated streptavidin. TMB substrate is used for color development at 450 nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of human Factor V. Color development is proportional to the concentration of Factor V in the samples.

Standard Calibration Factor V standard provided is calibrated against the WHO 1st International Standard for Factor V, Plasma, Human distributed by NIBSC (03/116), South Mimms, Potters Bar, Hertfordshire, UK.

Lot 912L-A: 10 ug = 0.79 IU

Reagents Provided:

- **96-well microtiter strip plate (8X12 removable wells):**

Containing anti-human Factor V monoclonal antibody dried and blocked on the surface

- **10X Wash Buffer:**

1 bottle of 50 ml; bring to 1X using DI water

- **Human Factor V standard:**

1 vial of lyophilized standard

- **Anti-human Factor V primary antibody**

1 vial of lyophilized monoclonal antibody

- **HRP-streptavidin:**

1 vial of concentrated HRP labeled streptavidin

- **TMB substrate solution:**

1 bottle of 10 ml solution

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Storage and Stability:

All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -80°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 0-10 µl and 200-1000 µl
- 12-channel pipette covering 30-300 µl
- Paper towels or kimwipes
- 50 ml tubes, 1.5 ml centrifuge tubes
- 1 N H_2SO_4
- Magnetic stirrer and stir-bars
- DI water
- Plastic containers with lids
- Microtiter plate spectrophotometer operable at 450 nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm. (Optional)

Warnings:

Warning – Avoid skin and eye contact when using TMB 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 substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the 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 V in the 0.5-500 ng/ml range. Samples giving human Factor V levels above 500ng/ml should be diluted in blocking buffer before use. A 1:100 to 1:500 dilution for plasma is suggested for best results.

Assay Procedure:

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

Preparation of Standard:

Reconstitute standard with 100 µl of deionized water. Add 900 µl of blocking buffer and mix thoroughly to give a 10 µg/ml solution. Add 100µl of the 10 µg/ml solution to 900 µl of blocking buffer and mix thoroughly to give a 1000 ng/ml solution.

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Dilution table for preparation of human Factor V standards:

Factor V concentration (ng/ml)	Dilutions
500	500µl (BB) + 500µl (1000ng/ml)
200	600µl (BB) + 400µl (500ng/ml)
100	500µl (BB) + 500µl (200ng/ml)
50	500µl (BB) + 500µl (100ng/ml)
20	600µl (BB) + 400µl (50ng/ml)
10	500µl (BB) + 500µl (20ng/ml)
5	500µl (BB) + 500µl (10ng/ml)
2	600µl (BB) + 400µl (5ng/ml)
1	500µl (BB) + 500µl (2ng/ml)
0.5	500µl (BB) + 500µl (1ng/ml)
0	500µl (BSA) 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. 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 10ml of blocking buffer directly to the primary antibody vial and agitate gently to completely dissolve contents. Add 100µl 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.

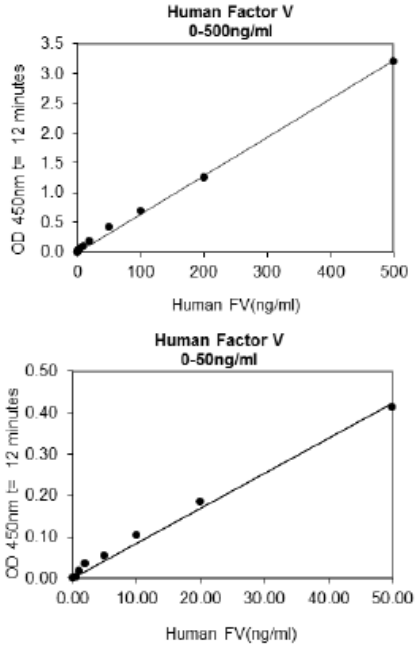
Secondary Reagent Addition:
Dilute 2.5 µl of HRP conjugated streptavidin into 2.5ml blocking buffer to generate a 1:1,000 dilution. Add 0.2 ml of 1:1,000 dilution to 9.8 ml of blocking buffer to generate a 1:50,000 dilution. Add 100 µl of the 1:50,000 dilution to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation:
Add 100 µl TMB substrate to all wells and shake plate for 2-5 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

Measurement:
Set the absorbance at 450 nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450 nm. 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 V in the standards. Fit a straight line through the points using a linear fit procedure. The amount of total human Factor V in the unknowns can be determined from this curve.

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



Expected Values: The concentration of Factor V in normal human plasma ranges from 6.6 to 8.25 µg/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 Kit Plate Layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.5ng/ml	1ng/ml	2ng/ml	5ng/ml	10ng/ml	20ng/ml	50ng/ml	100ng/ml	200ng/ml	500ng/ml	
B	0	0.5ng/ml	1ng/ml	2ng/ml	5ng/ml	10ng/ml	20ng/ml	50ng/ml	100ng/ml	200ng/ml	500ng/ml	
C												
D												
E												
F												
G												
H												

96 Well Plate
Standards: 22 Wells
Samples: 74 Wells

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

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