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Total Human Coagulation Factor XII Antigen Assay

Strip well format. Reagents for up to 96 tests

Catalog No.	**CS161A**	**1 x 96 tests**
	CS161B	**5 x 96 tests**

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

Background: Factor XII (aka Hageman Factor) is a single-chain, 615 amino acid glycoprotein zymogen. Factor XII is activated by kallikrein. Factor XIIa converts prekallikrein to kallikrein during the intrinsic pathway of the coagulation cascade. Although Factor XII is not thought to play an essential role in normal hemostasis, lack of Factor XII in a mouse model resulted in a 'severe defect' in thrombus formation.

Assay Principle: Human Factor XII will bind to the affinity purified capture antibody coated on the microtiter plate. Factor XII and XIIa will react with the antibody on the plate. After appropriate washing steps, anti-human Factor XII primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody, which is proportional to the total Factor XII present in the samples, is reacted with the secondary antibody. Following an additional washing step, 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 XII. Color development is proportional to the concentration of Factor XII in the samples.

Reagents Provided:

♦96-well microtiter strip plate
8X12 removable well strips containing affinity purified anti-human Factor XII antibody on the surface. Strips are blocked and dried.

♦10X Wash Buffer
1 bottle of 50 mL; bring to 1X using DI water

♦Human Factor XII standard
1 vial of lyophilized standard

♦Anti-human Factor XII primary antibody
1 vial of lyophilized polyclonal antibody

♦HRP-Secondary antibody
1 vial of concentrated HRP-labeled antibody

♦TMB substrate solution
1 bottle of 10 mL solution

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 -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.

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Reagents and Equipment Required:

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

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 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 XII in the 0.1-100 ng/mL range. Samples giving human Factor XII levels above 100ng/mL should be diluted in blocking buffer before use. A 1:1,000 to 1:5,000 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 as directed on the vial to give a 1,000 ng/mL solution.**

Dilution table for preparation of human Factor XII standards:

Factor XII concentration (ng/mL)	Dilutions
100	900µL (BB) + 100µL (1000ng/mL)
50	500µL (BB) + 500µL (100ng/mL)
25	500µL (BB) + 500µL (50ng/mL)
10	600µL (BB) + 400µL (25ng/mL)
5	500µL (BB) + 500µL (10ng/mL)
2.5	500µL (BB) + 500µL (5ng/mL)
1	600µL (BB) + 400µL (2.5ng/mL)
0.5	500µL (BB) + 500µL (1ng/mL)
0.25	500µL (BB) + 500µL (0.5ng/mL)
0.1	600µL (BB) + 400µL (0.25ng/mL)
0	500µL (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. Add 100 µL standards in duplicate and unknowns to wells. Carefully record position of standards and unknowns. 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.

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.

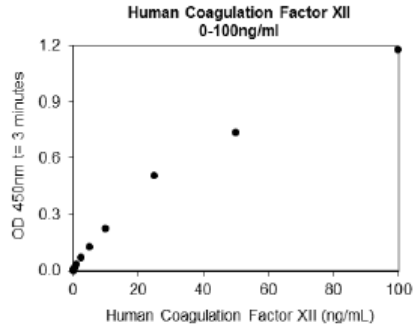
Secondary Antibody Addition:
Dilute 2 µL into 10 mL blocking buffer and add 100 µL to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 µL wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

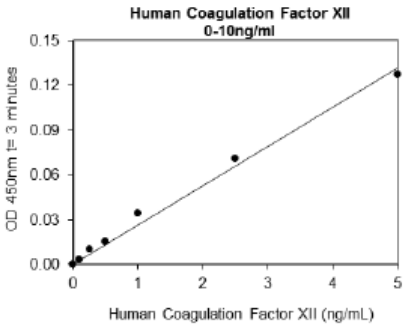
Substrate Incubation:
Add 100 µL TMB substrate to all wells and shake plate for 2-7 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50 µL of 1N H_2SO_4 stop solution to all wells when samples are visually in the same range as the standards. Add 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 XII in the standards. Fit a straight line through the points using a linear fit procedure. The amount of total human Factor XII in the unknowns can be determined from this curve.

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





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Expected Values: The concentration of Factor XII in normal human plasma has been found to be 29 µg/mL, with variation among individuals from 15 to 47 µg/mL. Another series of studies found values in the 35-40 µg/mL range.

Disclaimer: This information is believed to be correct but does not purport 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:
96 Well Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.1ng/mL	0.25ng/mL	0.5ng/mL	1 ng/mL	2.5 ng/mL	5 ng/mL	10 ng/mL	25 ng/mL	50 ng/mL	100ng/mL	
B	0	0.1ng/mL	0.25ng/mL	0.5ng/mL	1 ng/mL	2.5 ng/mL	5 ng/mL	10 ng/mL	25 ng/mL	50 ng/mL	100ng/mL	
C												
D												
E												
F												
G												
H												

Standards: 22 Wells
Samples: 74 Wells

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

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