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Rat Anti-Factor XII Total Antigen ELISA Kit

Catalog No.	CKR003A CKR003B	**Quantity:** **Quantity:**	1 x 96 tests 5 x 96 tests

Intended Use: This rat coagulation Factor XII antigen assay is intended for the quantitative determination of total Factor XII antigen in rat plasma. This kit has been formulated for research only.

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: Rat 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, biotin labeled anti-rat Factor XII primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody is reacted with peroxidase conjugated streptavidin. 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 rat Factor XII. Color development is proportional to the concentration of Factor XII in the sample.

Reagents Provided:
A: 96-well antibody coated microtiter strip plate (removable strips 8x12):
containing anti-Rat Factor XII antibody dried and blocked on the strip well surface
B: 10X Wash Buffer: 1 bottle of 50 ml; bring to 1X using DI water
C: Rat Facotr XII standard: 1 vial lyophilized standard
D: Biotinylated anti-Rat Factor XII detection antibody: 1 vial lyophilized anti-Rat Factor XII polyclonal antibody
E: Horseradish peroxidase streptavidin: 1 vial concentrated HRP labeled streptavidin
F: TMB substrate solution: 1 bottle 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 -80°C for later use. DO NOT freeze/thaw the standard and primary antibody more than once. All other unused kit components must be stored at 4°C. The kit should be used no later than the expiration date.

Reagents and Equipment

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement

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Required:

- 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)

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.
- All kit components must be kept refrigerated (4°C).
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

Preparation of Reagents:

TBS buffer: 0.1 M TRIS, 0.15 M NaCl, pH 7.4

Blocking buffer (BB): 3% BSA (w/v) in TBS buffer

1 x Wash buffer: Dilute 50 ml of 10X wash buffer concentrate with 450 ml deionized water.

Specimen Collection:

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for at least 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store at $\leq$-20°C. Avoid repeated freeze-thaw cycles.

Assay Procedure:

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

Preparation of Standard:

Reconstitute standard by adding 10 ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 100 ng/ml standard solution.

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Table 1: Dilution table for preparation of Human Factor XII standard:

Factor XII Concentration (ng/ml)	Dilutions
10	900 µl (BB) + 100 µl (100 ng/ml)
5	500 µl (BB) + 500 µl (10 ng/ml)
2	600 µl (BB) + 400 µl (5 ng/ml)
1	500 µl (BB) + 500 µl (2 ng/ml)
0.5	500 µl (BB) + 500 µl (1 ng/ml)
0.2	600 µl (BB) + 400 µl (0.5 ng/ml)
0.1	500 µl (BB) + 500 µl (0.2 ng/ml)
0.05	500 µl (BB) + 500 µl (0.1 ng/ml)
0.02	600 µl (BB) + 400 µl (0.05 ng/ml)
0	500 µl (BB) Zero point to determine background

NOTE: Dilutions for the standard curve must be made and applied to the plate immediately.

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

Standard and Unknown Addition:
Remove microtiter plate from bag and add 100 µl Factor XII 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.

NOTE: The assay measures Factor XII antigen in the 0.02 -10 ng/ml range. If the unknown is thought to have high Factor XII levels, dilutions may be made in blocking buffer. A 1:10,000-1:100,000 dilution for normal rat plasma is suggested for best results.

Detection Antibody Addition:
Reconstitute biotinylated detection antibody by adding 10 ml of blocking buffer directly to the 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.

Streptavidin-HRP Addition:
Briefly centrifuge vial before opening. Dilute 2.5 µl of HRP conjugated streptavidin into 2.5 ml blocking buffer to generate a 1:1,000 dilution. Add 0.4 ml of the 1:1,000 dilution to 9.6 ml of blocking buffer to generate a 1:25,000 dilution. Add 100 µl of the 1:25,000 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 2-7 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50 µl of 1N H_2SO_4 of 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 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}).

Calculation of Results:
Plot A_{450} against the amount of Factor XII in the standards. Fit a straight line through the points 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 XII in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

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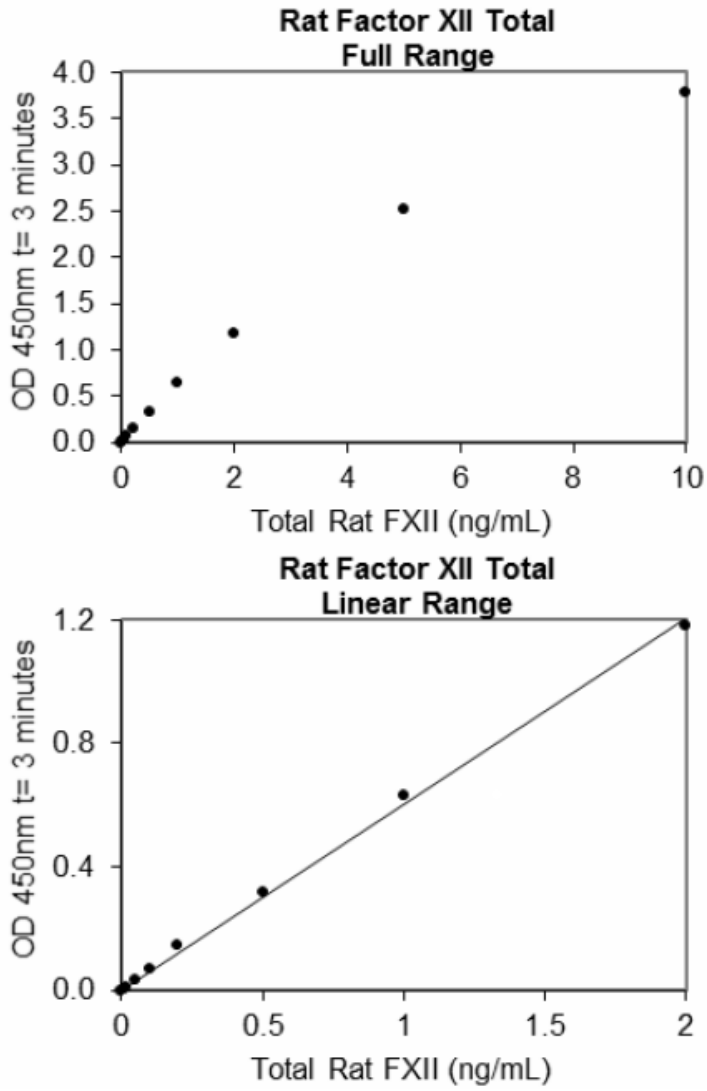
A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)



Expected Values: The concentration of Factor XII in normal human plasma has been found to be approximately 30 µg/ml, with variation among individuals from 15 to 45 µg/ml. Normal values of Factor XII in rat plasma have not been conclusively determined but are believed to be similar to human plasma.

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: 0.091-0.105) and calculating the corresponding concentration. The MDD was 0.0147 ng/ml.

Specificity: This assay recognizes natural and recombinant rat Factor XII and Factor XIIa. Pooled normal plasma from dog, rabbit, cynomolgus monkey, rhesus monkey, pig, and sheep were assayed and no significant cross-reactivity was observed. Pooled normal plasma from human was assayed and minor cross-reactivity was observed. Significant cross-reaction is observed with pooled normal plasma from mouse.

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

Sample Type	Dilution	Mean (µg/mL)
Citrate Plasma	1:50,000	27.6
	1:100,000	28.0

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

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