

INTENDED USE

This mouse coagulation Factor XII antigen assay is intended for the quantitative determination of total Factor XII antigen in mouse plasma. **For research use only.**

BACKGROUND

Factor XII (aka Hageman Factor) is a single-chain, 615 amino acid glycoprotein zymogen [1]. Factor XII is activated by kallikrein [2]. 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 [3].

ASSAY PRINCIPLE

Mouse 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-mouse 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 mouse Factor XII. Color development is proportional to the concentration of Factor XII in the samples.

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-mouse Factor XII antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Mouse Factor XII standard:** 1 vial lyophilized standard
- **Anti-mouse Factor XII primary antibody:** 1 vial lyophilized polyclonal antibody
- **Horseradish peroxidase-conjugated streptavidin:** 1 vial concentrated HRP labeled streptavidin
- **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 standard 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 H₂SO₄ 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 1ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 1000ng/ml standard solution.

Make an intermediate dilution of 100ng/ml by adding 100 μl of the 1000ng/ml standard solution to 900 μl of blocking buffer.

Dilution table for preparation of Factor XII standard:

Factor XII concentration (ng/ml)	Dilutions
10	900 μl (BB) + 100 μl (100ng/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)
0.1	500 μl (BB) + 500 μl (0.2ng/ml)
0.05	500 μl (BB) + 500 μl (0.1ng/ml)
0.02	600 μl (BB) + 400 μl (0.05ng/ml)
0.01	500 μl (BB) + 500 μl (0.02ng/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 XII 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 XII antigen in the 0.01-10 ng/ml range. If the unknown is thought to have high Factor XII levels, dilutions may be made in blocking buffer. A 1:50,000-1:400,000 dilution for normal mouse plasma is suggested for best results.

Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. 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.

Streptavidin-HRP Addition

Briefly centrifuge vial before opening. Dilute 2.5 μl of HRP conjugated streptavidin into 2.5ml blocking buffer to generate a 1:1,000 dilution. Add 0.4ml of the 1:1,000 dilution to 9.6ml 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 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 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.

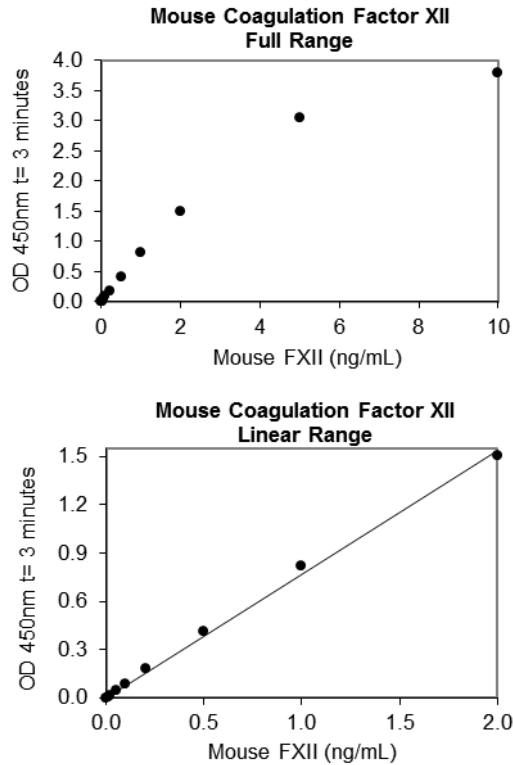
Measurement

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 (A_{450}).

Calculation of Results

Plot A_{450} against the amount of Factor XII 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 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.

A typical standard curve (EXAMPLE ONLY):



EXPECTED VALUES

The concentration of Factor XII in normal human plasma has been found to be 29 $\mu\text{g/mL}$, with variation among individuals from 15 to 47 $\mu\text{g/mL}$ [1]. Another series of studies found values in the 35-40 $\mu\text{g/mL}$ range [4,5]. Normal values of Factor XII in mouse plasma have not been conclusively determined but are believed to be similar to human plasma based on in-house testing.

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: 0.058-0.064) and calculating the corresponding concentration. The MDD was 0.005 ng/ml.

Intra-assay Precision: Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Sample	1	2	3
n	20	20	20
Mean (ng/ml)	0.055	0.29	0.825
Standard Deviation	0.0034	0.0084	0.014
CV (%)	6.25	2.88	1.71

Inter-assay Precision: Three samples of known concentration were tested in ten independent assays to assess inter-assay precision.

Sample	1	2	3
n	10	10	10
Mean (ng/ml)	0.238	0.725	2.040
Standard Deviation	0.021	0.040	0.126
CV (%)	9.04	5.52	6.19

Recovery: The recovery of antigen spiked to levels throughout the range of the assay in blocking buffer was evaluated.

Sample	1	2	3	4
n	4	4	4	4
Mean (ng/ml)	0.125	0.3	3.0	7.5
Average % Recovery	98	93	95	99
Range	94-102%	91-95%	92-96%	90-105%

Linearity: To assess the linearity of the assay, pooled citrated mouse plasma samples containing high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay.

Sample	1:2	1:4	1:8	1:16
n	4	4	4	4
Average % of Expected	102	101	101	102
Range	101-103%	99-104%	100-103%	95-111%

Specificity: This assay recognizes natural and recombinant mouse Factor XII and Factor XIIa. Pooled normal plasma from rat, rabbit, human, pig, dog, cyno monkey, rhesus monkey and sheep was assayed and no significant cross-reactivity was observed.

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

Sample Type	Dilution	Mean (µg/mL)
Citrate Plasma	1:100,000	26.6
	1:200,000	27.8
	1:400,000	27.3

Example of ELISA Plate Layout
96 Well Plate: 22 Standard wells, 74 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.01 ng/ml	0.02 ng/ml	0.05 ng/ml	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	
B	0	0.01 ng/ml	0.02 ng/ml	0.05 ng/ml	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	
C												
D												
E												
F												
G												
H												

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. Revak SD, *et al.*: J Clin Invest. 1974, 54(3):619-627.
2. Meier HL, *et al.*: J Cli. Invest. 1977, 60(1):18-31.
3. Renné T, *et al.*: J Exp Med. 2005, 202(2):271-281.
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5. Saito H: Adv Exp Med Biol. 1979, 120A:165-172.