cellsciences.com

# Mouse Coagulation Factor XII Total Antigen ELISA Kit

Strip well format. Reagents for up to 96 tests

| | | |
|---|---|---|
| **Catalog No.** | **CKM022A** | **1 x 96 tests** |
| | **CKM022B** | **5 x 96 tests** |

**Intended Use:** This mouse coagulation Factor XII antigen assay is intended for the quantitative determination of total Factor XII antigen in mouse 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:** 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, anti-mouse 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 mouse Factor XII. Color development is proportional to the concentration of Factor XII in the samples.

**Reagents Provided:**

♦ **96-well microtiter strip plate**
8 x 12 removable well strips containing affinity purified anti-mouse 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

♦ **Mouse Factor XII standard**
1 vial of lyophilized standard

♦ **Anti-mouse 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

**Cell Sciences®**
Neponset Valley Tech Park
480 Neponset St., Bldg. 12A
Canton, MA 02021

Toll Free: 888 769-1246
Phone: 781 828-0610
Fax: 781 828-0542

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

**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. The kit should not be used beyond the expiration date.

**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
- Polypropylene tubes for dilution of standard
- 1 N $H_2SO_4$
- Deionized water
- Bovine Serum Albumin Fraction V (BSA)
- Tris (hydroxymethyl)aminomethane (Tris)
- NaCl
- 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
- Manifold dispenser/aspirator or automated microplate washer

**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.
- 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** 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: 0.1 M Tris 0.15 M NaCl pH 7.4
- Blocking buffer (BB): 3% BSA in TBS
- Wash buffer concentrate: The wash buffer supplied in a 10X concentrate must be diluted 1:10 with deionized water for use with the kit.

**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 ≤ -20°C. **Avoid repeated freeze-thaw cycles.**

**Cell Sciences®**
Neponset Valley Tech Park
480 Neponset St., Bldg. 12A
Canton, MA 02021

Toll Free: 888 769-1246
Phone: 781 828-0610
Fax: 781 828-0542

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

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

Dilution table for preparation of mouse Factor XII standards:

| Factor XII concentration (ng/ml) | Dilutions |
|---|---|
| 10 | 900µl (BB) + 100µl (100ng/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) |
| 0.2 | 600µl (BB) + 400µl (0.5ng/ml) |
| 0.1 | 500µl (BB) + 500µl (0.2ng/ml) |
| 0.05 | 500µl (BB) + 500µl (0.1ng/ml) |
| 0.02 | 600µl (BB) + 400µl (0.05ng/ml) |
| 0.01 | 500µl (BB) + 500µl (0.02ng/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.

Note: The assay measures Factor XII antigen in the 0.01–10 ng/ml range. If the unknowns are thought to have high Factor XII levels, dilutions may be made in blocking buffer. A 1:50,000 to 1:400,000 dilution for normal mouse plasma is recommended for best results.

**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.

**Streptavidin-HRP Addition:** 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 9.6ml 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 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 1 N $H_2SO_4$ 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 ($A_{450}$).

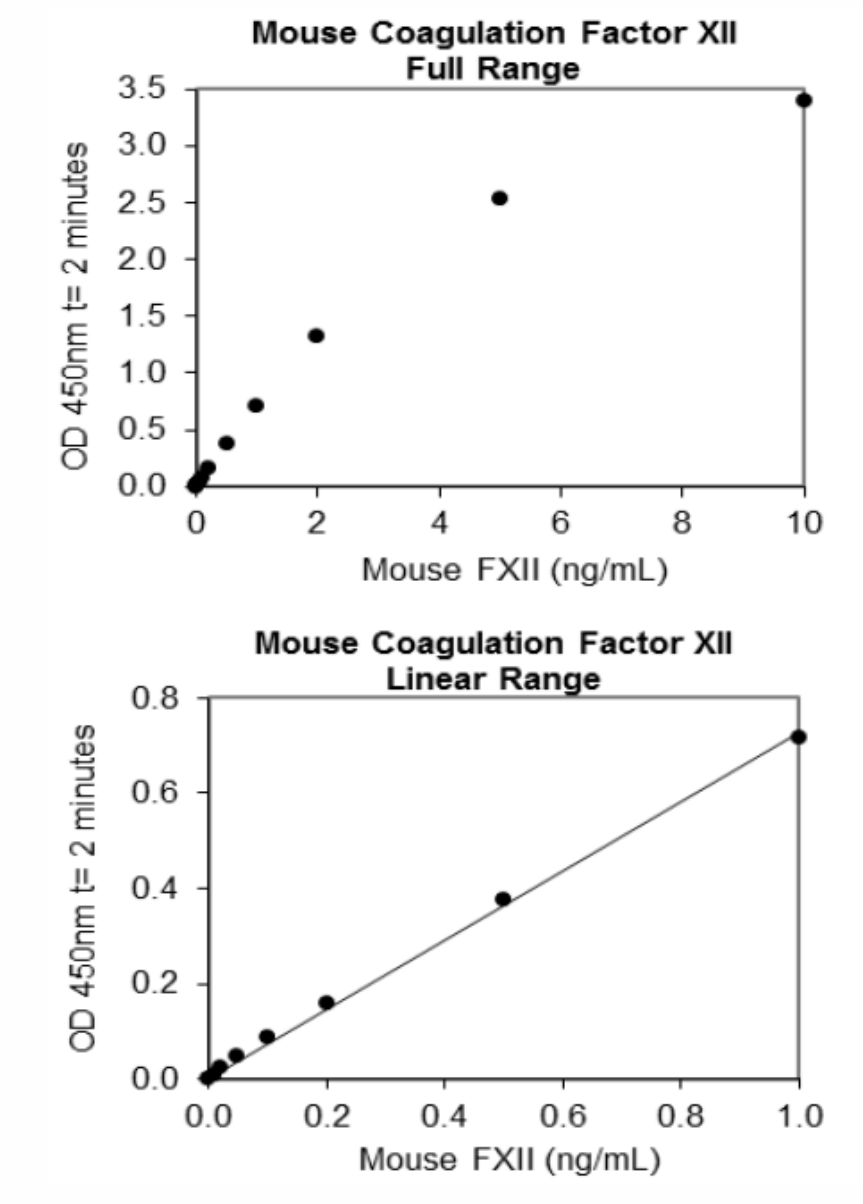
**Calculation of Results:** Plot A450 against the amount of Factor XII in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing 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.

**Cell Sciences®**
Neponset Valley Tech Park
480 Neponset St., Bldg. 12A
Canton, MA 02021

Toll Free: 888 769-1246
Phone: 781 828-0610
Fax: 781 828-0542

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

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




**Cell Sciences**®
Neponset Valley Tech Park
480 Neponset St., Bldg. 12A
Canton, MA 02021

Toll Free: 888 769-1246
Phone: 781 828-0610
Fax: 781 828-0542

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

**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. 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 20 zero standard replicates (range OD450: 0.058-0.064) and calculating the corresponding concentration. The MDD was 0.0046 ng/ml.

**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% |

**Cell Sciences®**
Neponset Valley Tech Park
480 Neponset St., Bldg. 12A
Canton, MA 02021

Toll Free: 888 769-1246
Phone: 781 828-0610
Fax: 781 828-0542

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

**Specificity:** This assay recognizes natural and recombinant mouse Factor XII and Factor XIIa. Pooled normal plasma from Rat, Rabbit, Human, Pig, and Sheep were 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 |

**Cell Sciences®**
Neponset Valley Tech Park
480 Neponset St., Bldg. 12A
Canton, MA 02021

Toll Free: 888 769-1246
Phone: 781 828-0610
Fax: 781 828-0542

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

**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**
**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 | | | | | | | | | | | | |

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

**Cell Sciences®**
Neponset Valley Tech Park
480 Neponset St., Bldg. 12A
Canton, MA 02021

Toll Free: 888 769-1246
Phone: 781 828-0610
Fax: 781 828-0542

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com