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Mouse Prothrombin Total Antigen ELISA Kit

Strip well format. Reagents for up to 96 tests

Catalog No.	**CS455A**	**Quantity:**	**1 X 96 tests**
	CS455B		**5 x 96 tests**

Intended Use: This mouse prothrombin antigen assay is intended for the quantitative determination of total prothrombin antigen in mouse plasma

Background: Prothrombin (aka Factor II) is a single chain vitamin K dependent 579 amino acid glycoprotein zymogen. Prothrombin is proteolytically activated to thrombin by the prothrombinase enzyme complex in the coagulation cascade common pathway. The serine protease thrombin converts plasma fibrinogen to insoluble fibrin. Prothrombin levels are decreased by anticoagulant therapy, vitamin K deficiency and severe liver disease. Elevated plasma prothrombin is associated with a single nucleotide change at position 20210.

Assay Principle: Mouse prothrombin will bind to the capture antibody coated on the microtiter plate. Prothrombin, thrombin, and thrombin/anti-thrombin complex 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 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of prothrombin. The amount of color development is directly proportional to the concentration of prothrombin in the sample.

Reagents Provided:

- **96-well microtiter strip plate:**
  8X12 removable well strips containing anti-mouse prothrombin antibody on the surface. Strips are blocked and dried.
- **10X Wash Buffer:**
  1 bottle of 50 ml; bring to 1x using DI water
- **5x Diluent**
  1 bottle of 50 ml; bring to 1x using DI water
- **Mouse prothrombin standard:**
  1 vial of lyophilized standard
- **Anti-mouse prothrombin primary antibody:**
  1 vial of lyophilized polyclonal antibody
- **HRP streptavidin:**
  1 vial concentrated HRP labeled streptavidin.
- **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 -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.

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Reagents and Equipment Required:

- 1-channel pipettes covering 20-200 µl, 200-1000 µl and 500-5000µl
- 12-channel pipette for 30-300µl
- Paper towels or kimwipes
- 1.5 ml micro centrifuge tubes
- 1N H_2SO_4
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- TBS buffer
- Blocking buffer
- 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 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:** 0.1 M Tris, 0.15 M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA in TBS
- **The Diluent concentrate:** The Diluent supplied in a 5X concentrate must be brought to room temperature so all the precipitate is dissolved and then diluted 1:5 with deionized water to be used in the kit.
- **Wash buffer concentrate:** The wash buffer supplied is in a 10X concentrate and must be diluted 1:10 with deionized water for use with the kit.

Specimen Preparation:

The assay measures total mouse prothrombin in the 1-500 ng/ml range. Samples giving prothrombin levels above 500 ng/ml should be diluted in blocking buffer before use.
A dilution of at least 1:10,000 is recommended for measurement of prothrombin in normal mouse plasma.
Samples of mouse serum, tissue extracts and cell culture media may be applied directly to the plate.

Assay Procedure:

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

Preparation of Standard: Reconstitute 1000 ng standard vial with 1.0 ml of blocking buffer to give a 1000 ng/ml stock solution.

Dilution table for preparation of mouse prothrombin standard curve:

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Prothrombin concentration (ng/mL)	Dilutions
500	500µl (Diluent) + 500µl (1,000ng/mL)
200	600µl (Diluent) + 400µl (500ng/mL)
100	500µl (Diluent)+ 500µl (200ng/mL)
50	500µl (Diluent) + 500µl (100ng/mL)
20	600µl (Diluent) + 400µl (50ng/mL)
10	500µl (Diluent) + 500µl (20ng/mL)
5	500µl (Diluent) + 500µl (10ng/mL)
2	600µl (Diluent) + 400µl (5ng/mL)
1	500µl (Diluent) + 500µl (2ng/mL)
0	500µl (Diluent) 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 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.

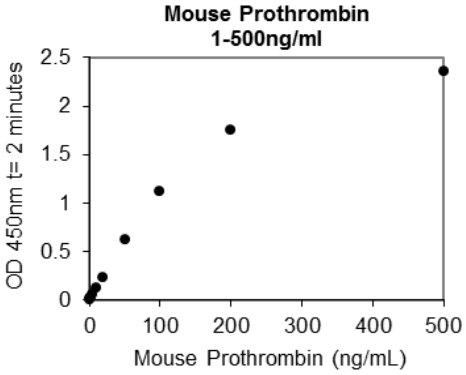
Secondary Antibody Addition:
Dilute 2.5 µl of HRP conjugated streptavidin into 2.5 ml blocking buffer to generate a 1:1,000 dilution. Add 0.1 ml of 1:1,000 dilution to 9.9ml of blocking buffer to generate a 1:100,000 dilution. Add 100 µl of the 1:100,000 dilution to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

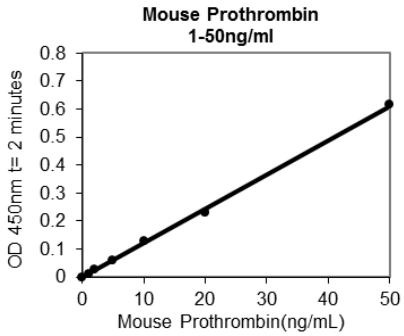
Substrate Incubation:
Add 100 µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50 µl of 1N H_2SO_4 stop solution to all wells when samples are visually in the same range as the standards. Add 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 prothrombin in the standards. Fit a straight line through the linear points of the standard curve 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 total mouse prothrombin 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, DO NOT USE)





Expected Values: Prothrombin in normal human plasma ranges from 110-212 µg/ml with an average concentration of 150 µg/ml. Normal values of prothrombin in mouse plasma have not been conclusively determined but are believed to be similar to human plasma.

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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 and calculating the corresponding concentration. The MDD was 0.319 ng/mL.

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

	Intra-assay Precision		
Sample	1	2	3
n	20	20	20
Mean (ng/mL)	2.81	14.1	39.5
Standard Deviation	0.267	0.560	1.40
CV (%)	9.51	3.98	3.53

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

	Inter-assay Precision		
Sample	1	2	3
n	10	10	10
Mean (ng/ml)	2.42	13.8	145
Standard Deviation	0.41	1.20	7.18
CV (%)	9.96	8.72	4.95

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)	2.53	5.26	9.89	40.5
Average % Recovery	101	96	94	90
Range	95-106%	86-105%	89-97%	86-93%

Linearity: To assess the linearity of the assay, human 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	98	90	92	111
Range	93-103%	75-99%	84-109%	99-137%

Specificity: This assay recognizes natural mouse total prothrombin (prothrombin, thrombin, and thrombin-antithrombin complex). This assay cross-reacts with rat prothrombin. Pooled normal plasma from the species listed below were assayed for cross reactivity. No significant cross-reactivity was observed.

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Natural human prothrombin
Natural porcine prothrombin
Natural rabbit prothrombin
Natural sheep prothrombin

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

Sample Type	Dilution	Mean (µg/mL)
Citrate Plasma	1:10,000	216
	1:20,000	199
	1:40,000	201

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 96 Well Plate Layout

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

Standards: 20 Wells
Samples: 76 Wells

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

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