Catalog # MPTKT

Mouse Prothrombin ELISA

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

This prothrombin assay is intended for the quantitative determination of mouse prothrombin in biological fluids. The assay will not detect mouse thrombin.

BACKGROUND

Prothrombin (aka Factor II) is a single-chain vitamin K dependent 579 amino acid glycoprotein zymogen [1]. 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 [2]. Elevated plasma prothrombin is associated with a single nucleotide change at position 20210 [3].

ASSAY PRINCIPLE

Mouse prothrombin will bind to the capture antibody coated on the microtiter plate. Thrombin and thrombin-atithrombin complex will not react with 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 50mL; 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 of concentrated HRP-labeled streptavidin
- **TMB substrate solution**
  1 bottle of 10mL 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 standard, primary and secondary may be stored at -70°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. Kit should be used no later than the expiration date.

REAGENTS AND EQUIPMENT REQUIRED

- 1-channel pipettes covering 20-200 µl, 500-5000 µl and 200-1000µl
- 12-channel pipette for 30-300µl
- Paper towels or kimwipes
- 1.5ml 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 450nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300rpm

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 PREPARATION

The assay measures mouse prothrombin in the 1-500ng/ml range. Samples giving prothrombin levels above 500ng/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 (300rpm) at each step of the assay.

Preparation of Standard:
Reconstitute standard as directed on the vial to give a 1,000ng/mL solution.

Dilution table for preparation of mouse prothrombin standards:

Prothrombin concentration (ng/mL)	Dilutions
500	500µl (BB) + 500µl (1,000ng/mL)
200	600µl (BB) + 400µl (500ng/mL)
100	500µl (BB)+ 500µl (200ng/mL)
50	500µl (BB) + 500µl (100ng/mL)
20	600µl (BB) + 400µl (50ng/mL)
10	500µl (BB) + 500µl (20ng/mL)
5	500µl (BB) + 500µl (10ng/mL)
2	600µl (BB) + 400µl (5ng/mL)
1	500µl (BB) + 500µl (2ng/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 100µl 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 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Primary Antibody Addition:
Add 10ml of blocking buffer directly to the primary antibody vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

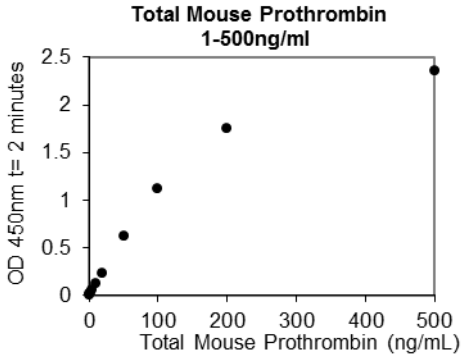
Secondary Antibody Addition:
Dilute 2.5µl of HRP conjugated streptavidin into 2.5ml blocking buffer to generate a 1:1,000 dilution. Add 0.2ml of 1:1,000 dilution to 9.8ml of blocking buffer to generate a 1:50,000 dilution. Add 100µl of the 1:50,000 dilution to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

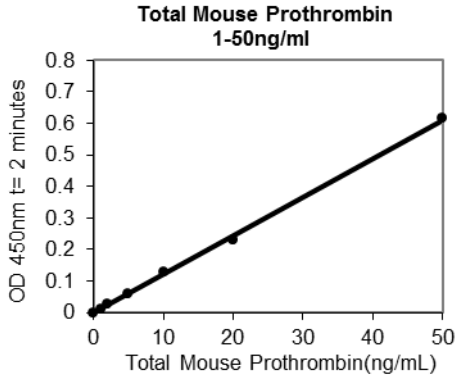
Substrate Incubation:
Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 stop solution to all wells when samples are visually in the same range as the standards. Add stop 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 450nm. For best results read plate immediately.

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}).

Assay Calibration:
Plot A_{450} against the amount of prothrombin in the standards. Fit a straight line through the points using a linear fit procedure. The prothrombin concentration of the unknowns can be determined by from this curve.

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 [4]. Normal values of prothrombin in mouse plasma have not been conclusively determined but are believed to be similar to human plasma.

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.

REFERENCES

1. KG Mann: Prothrombin. Methods Enzymol., **45**(Pt B):123-156, 1976.
2. A.D.A.M. Medical Encyclopedia [Internet]. Atlanta (GA): A.D.A.M., Inc.; c1997-2011. Factor II deficiency; [last reviewed 2011 Feb 28; cited 2012].
3. Poort, SR *et al*.: A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood, **88**:3698-3703, 1996.
4. McDuffie, FC *et al*.: Prothrombin, thrombin and prothrombin fragments in plasma of normal individuals and of patients with laboratory evidence of disseminated intravascular coagulation. Thromb. Res., **16**:759-773, 1979.

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