Mouse Factor X Total Antigen Assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

This mouse Factor X total assay is for the quantitative determination of total Factor X and Xa in biological fluids.

BACKGROUND

Factor X is a disulfide linked two-chain glycoprotein zymogen and is the precursor of the coagulation enzyme Factor Xa [1]. Factor X serves as the intersection of the intrinsic and extrinsic coagulation cascades and can be activated by either the extrinsic Factor VIIa / Tissue Factor compex or the intrinsic Factor IXa / Factor VIIIa complex. Factor Xa converts prothrombin to thrombin and is quickly inhibited by Antithrombin III in the presence of heparin.

ASSAY PRINCIPLE

Mouse Factor X will bind to the affinity purified capture antibody coated on the microtiter plate. Factor X, Xa, and Xa in complex with inhibitors will react with the antibody on the plate. After appropriate washing steps, biotin labeled polyclonal anti-mouse Factor X primary antibody binds to the Factor X. Excess antibody is washed away and bound polyclonal antibody is then reacted with avidin conjugated to horseradish peroxidase. Following an additional washing step, TMB substrate is used for color development at 450nm. The amount of color development is directly proportional to the concentration of total Factor X in the sample.

REAGENTS PROVIDED

♦ **96-well antibody coated microtiter strip plate:** containing affinity purified sheep anti-mouse Factor X antibody dried and blocked on the strip well surface.

♦ **10X Wash Buffer:**
1 bottle of 50ml; bring to 1X using DI water

♦ **Mouse Factor X activity standard:**
1 vial of lyophilized recombinant standard

♦ **Anti-Factor X primary antibody:**
1 vial of lyophilized biotin labeled polyclonal rabbit anti-mouse antibody

♦ **Avidin peroxidase secondary reagent:**
1 vial of concentrated HRP labeled avidin

♦ **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 standards and primary may be stored at -70°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.

REAGENTS AND EQUIPMENT REQUIRED

- Pipettes covering 0-10µl and 200-1000µl
- 12-channel pipette covering 30-300µl
- Paper towels or kimwipes
- 50ml tubes
- 1N H_2SO_4

•DI water
•Magnetic stirrer and stir-bars
•Plastic containers
•ELISA plate cover
•TBS buffer
•3% BSA 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.
• All kit components must be stored at the correct temperatures.
• **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

•**TBS buffer:** 0.1M Tris-HCl 0.15M NaCl, pH 7.4
•**Blocking buffer (BSA):** 3% BSA in TBS buffer

SPECIMEN COLLECTION

Samples of mouse plasma in citrate, serum, urine, cell culture media, or tissue extracts may be applied directly to the plate.

NOTE: Plasma should be collected in citrate anticoagulant. Heparin or EDTA are not recommended. Heparin binds Factor X thus interfering with the assay.

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 vial to give a 1,000 ng/mL standard solution.

Dilution table for preparation of mouse factor X standards:

Factor X concentration (ng/mL)	Dilutions
1000	100µl from standard vial
500	500µL (BSA) + 500µL (1000ng/mL)
200	600µL (BSA) + 400µL (500ng/mL)
100	500µL (BSA) + 500µL (200ng/mL)
50	500µL (BSA) + 500µL (100ng/mL)
25	500µL (BSA) + 500µL (50ng/mL)
10	600µL (BSA) + 400µL (25ng/mL)
5	500µL (BSA) + 500µL (10ng/mL)
2.5	500µL (BSA) + 500µL (5ng/mL)
0	500µL (BSA) 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:
Add 100µl of Factor X 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:
Reconstitute primary antibody as directed on 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.

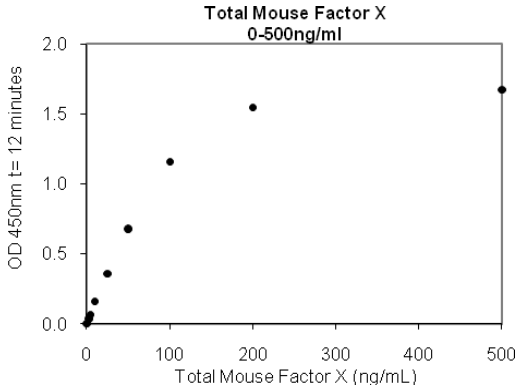
Secondary Reagent Addition:
Dilute 2.5µl conjugated secondary reagent in 10ml of 3% BSA blocking buffer and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

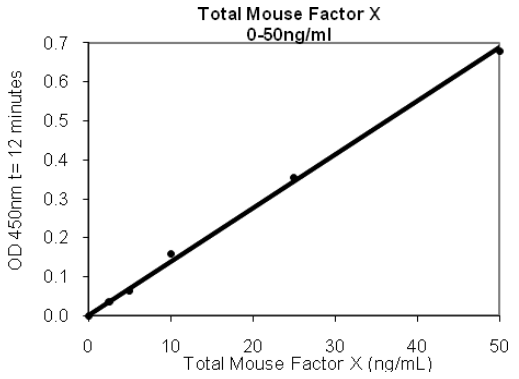
Substrate Incubation:
Add 100µl TMB substrate to all wells and shake plate for 10-20 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 stop solution to all wells when samples are visually in the same range as the standards. Add 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 Factor X in the standards. Fit a straight line through the points using a linear fit procedure. The Factor X in the unknowns can be determined from this curve.

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





EXPECTED VALUES

The concentration of human Factor X in normal plasma was found to be 10 µg/ml [2]. Normal values of Factor X in mouse plasma have not been conclusively determined but are believed to be similar to human plasma. Oral anticoagulants such as warfarin reduce functional Vitamin K and disrupt the post-translational addition of gamma-carboxyglutamic acid (gla) residues, decreasing the thrombotic activity of Factor Xa but not the concentration of Factor X antigen (3).

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. Di Scipio RG, *et al.*: A comparison of human prothrombin, factor IX (Christmas factor), factor X (Stuart factor), and protein S. Biochemistry, February 22, 1977; 16(4): 698-706.

2. Berthier AM, *et al.*: Comparison of immunological (ELISA) and biological determination of factor X in oral anticoagulant therapy. Haemostasis 12, 1982; 142.

3. Kumar S, *et al.*: Effect of warfarin on plasma concentrations of vitamin K dependent coagulation factors in patients with stable control and monitored compliance. Br J Haematol, January 1, 1990; 74(1): 82-5.

Example of 96 Well Plate Layout
Standards: 20 Wells
Samples: 76 Wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	2.5ng/ml	5ng/ml	10ng/ml	25ng/ml	50ng/ml	100ng/ml	200ng/ml	500ng/ml	1000ng/ml		
B	0	2.5ng/ml	5ng/ml	10ng/ml	25ng/ml	50ng/ml	100ng/ml	200ng/ml	500ng/ml	1000ng/ml		
C												
D												
E												
F												
G												
H												