Mouse Factor X Total Antigen ELISA

For Research Use Only

INTRODUCTION

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

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 complex 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.

PRINCIPLES OF PROCEDURE

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 HRP. After additional washing, TMB substrate is used for color development at 450 nm. The amount of color development is directly proportional to the concentration of total Factor X in the sample.

MATERIALS PROVIDED

Component	Contents	Quantity	Storage	Cat. No.
Coated Plate	Sheep Anti-Mouse Factor X coated 96-well plate	1 plate	4°C	CF41a
Standard	Lyophilized Mouse Factor X standard	1 vial	4°C	CF41b
Wash Buffer	10x solution for washing plate	50 mL	4°C	CF41c
Primary Antibody	Lyophilized Anti-Factor X-Biotin Conjugate	1 vial	4°C	CF41d
Streptavidin	HRP labeled Streptavidin	1 vial	4°C	CF41e
Substrate	TMB Substrate	10 mL	4°C	CF41f

MATERIALS NEEDED BUT NOT PROVIDED

1. Pipettes covering 0-10 μl and 200-1000 μl and tips
2. 12-channel pipette covering 30-300 μl
3. 1N H_2SO_4 or 1N HCl
4. DI water
5. Bovine Serum Albumin Fraction V (BSA)
6. TBS Buffer
7. Microtiter plate spectrophotometer with a 450 nm filter
8. Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm
9.

STORAGE CONDITIONS

1. Store this kit and its components at 4°C until use.
2. The reconstituted Standard and Primary Antibody may be stored at -70°C for later use. **DO NOT** freeze/thaw the Standard or Primary Antibody more than once.

PROCEDURAL NOTES

1. Use aseptic technique when opening and dispensing reagents.
2. This kit is designed to work properly as provided and instructed. Additions, deletions, or substitutions to the procedure or reagents are not recommended, as they may be detrimental to the assay.
3. Exercise universal precautions during the performance or handling of this kit or any component contained therein.

SAMPLE COLLECTION AND PREPARATION

Plasma should be collected using citrate as the anticoagulant. Heparin and EDTA are not recommended. Heparin binds Factor X and will interfere with the assay. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection. Assay immediately or store at -20°C. Avoid repeated freeze thaw cycles.

REAGENT PREPARATION

1. **10x Wash Buffer:** Dilute the 50 mL of concentrate to 1x with 450 mL of DI water prior to use.
2. **TBS Buffer:** 0.1 M Tris, 0.15 M NaCl, pH 7.4.
3. **Blocking Buffer:** 3% BSA in TBS Buffer.
4. **Standard:** Reconstitute standard by adding 1 mL of Blocking Buffer directly to the vial. Mix gently to dissolve contents. This will yield a 1,000 ng/mL standard solution.
5. **Primary Antibody:** Reconstitute the primary antibody by adding 10 mL of Blocking Buffer directly to the vial and mix gently until the contents are completely dissolved.

STANDARD PREPARATION

Prepare the Standard Stock Solution as described above. **Do not prepare the standards until you are ready to apply them to the plate.**

Table 1: Preparation of Standard Curve

Standard	Factor X Concentration (ng/mL)	Blocking Buffer (µL)	Transfer Volume (µL)	Transfer Source	Final Volume (µL)
S_8	500	500	500	Stock Vial	600
S_7	200	600	400	S_8	500
S_6	100	500	500	S_7	500
S_5	50	500	500	S_6	500
S_4	25	500	500	S_5	500
S_3	10	500	500	S_4	500
S_2	5	500	500	S_3	500
S_1	2.5	500	500	S_2	1000
S_0	0	500	---	---	500

ASSAY PROCEDURE

1. Add 100 μl of the Standards and unknowns to the wells in duplicate. Shake the plate at 300 rpm for 30 minutes at room temperature (RT). See Scheme I for a suggested plate layout.
2. Wash the plate 3 times according to the following wash procedure:
   a. Remove the contents of each well by inversion of the plate.
   b. Tap out the remaining contents of the plate onto a lint free paper towel.
   c. Add 300 μL of 1x Wash Buffer.
   d. Let stand for 1-2 minutes.
   e. Repeat procedure two more times, then proceed to step "f".
   f. Remove the contents of each well by inversion of plate into an appropriate disposal device.
   g. Tap out the remaining contents of the plate onto a lint free paper towel, then proceed to step 3.
3. Add 100 μl of the Primary Antibody to each well. Shake the plate at 300 rpm for 30 minutes at RT.
4. Wash the plate three times as in step 2.
5. Dilute 2.5 uL of Streptavidin-HRP conjugate in 2.5 mL of 1xTBS to make a 1:1000 dilution. Dilute 1.0 mL of the 1:1000 dilution to 9 mL to make a 1:10,000 dilution. Add 100 uL of the 1:10,000 dilution to all wells. Shake plate at 300 rpm for 30 minutes.
6. Wash the plate three times as in step 2.
7. Add 100 μl of TMB Substrate to each well. Shake the plate at 300 rpm for 1-5 minutes at RT.
8. Stop the reaction by adding 50 μl of 1N H_2SO_4 to each well and read the plate at 450 nm.

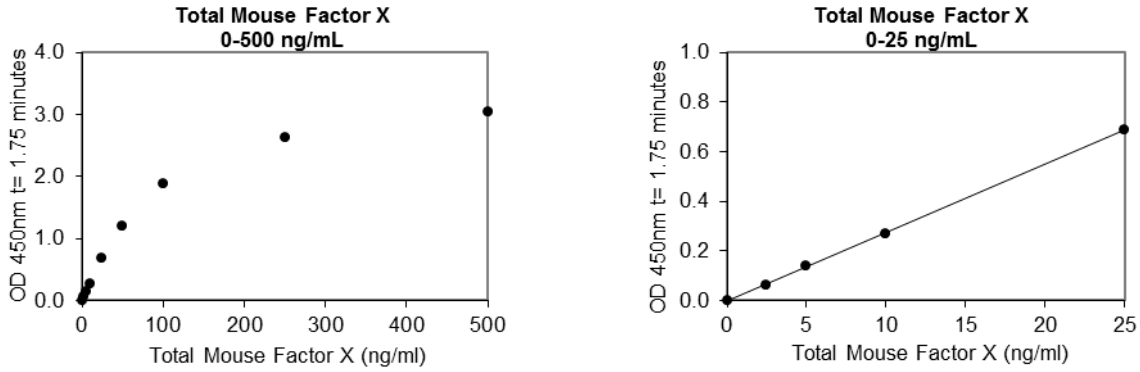
Scheme I: Suggested Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S_8	S_7	S_6	S_5	S_4	S_3	S_2	S_1	S_0	U_1	U_2	U_3
B	S_8	S_7	S_6	S_5	S_4	S_3	S_2	S_1	S_0	U_1	U_2	U_3
C	U_4	U_5	U_6	U_7	U_8	U_9	U_{10}	U_{11}	U_{12}	U_{13}	U_{14}	U_{15}
D	U_4	U_5	U_6	U_7	U_8	U_9	U_{10}	U_{11}	U_{12}	U_{13}	U_{14}	U_{15}
E	U_{16}	U_{17}	U_{18}	U_{19}	U_{20}	U_{21}	U_{22}	U_{23}	U_{24}	U_{25}	U_{26}	U_{27}
F	U_{16}	U_{17}	U_{18}	U_{19}	U_{20}	U_{21}	U_{22}	U_{23}	U_{24}	U_{25}	U_{26}	U_{27}
G	U_{28}	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	U_{37}	U_{38}	U_{39}
H	U_{28}	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	U_{37}	U_{38}	U_{39}

CALCULATIONS

1. Plot the A_{450} against the concentration of Factor X in the standards.
2. Fit a straight line through the points using a linear fit procedure.
3. Calculate the Factor X concentrations in the unknowns using the equation generated by the standard curve.

Figure 1: Typical Standard Curve



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2. Berthier AM, et al.: Haemostasis. 1982. 142
3. Kumar S, et al.: British Journal of Haematology. 1990 74(1): 82-5

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