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ab272779
Mouse FX ELISA kit (total FX antigen)

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For the quantitative determination of total Factor X and Xa in biological fluids.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Mouse FX ELISA kit (total FX antigen) is for the quantitative determination of total Factor X and Xa in biological fluids.

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 Streptavidin 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.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed.

Add 100 µL standard or sample to appropriate wells and shake plate at 300 rpm for 30 mins. Wash wells three times with 300 µL wash buffer. Remove excess wash by gently tapping plate on paper towel.

Add 100 µL of primary antibody to each well and shake plate at 300 rpm for 30 mins. Wash wells three times with 300 µL wash buffer. Remove excess wash by gently tapping plate on paper towel.

Add 100 µL Streptavidin-HRP to each well and shake plate at 300 rpm for 30 mins. Wash wells three times with 300 µL wash buffer. Remove excess wash by gently tapping plate on paper towel.

Add 100 µL TMB Substrate Solution to each well and shake plate for 2-10 mins.

Stop reaction with 50 µL H_2SO_4 or HCl stop solution, then measure the absorbance in all wells at 450nm.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Mouse Factor X ELISA Plate	1 x 96 tests	+4°C
10X Wash Buffer	1 x 50 mL	+4°C
Mouse Factor X Standard Lyophilized Vial	1 vial	+4°C
Anti-Mouse Factor X Primary Antibody Lyophilized Vial	1 vial	+4°C
Streptavidin-HRP Secondary Reagent	1 vial	+4°C
TMB Substrate	1 x 10 mL	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate plate shaker capable of 300 rpm uniform horizontal circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 TBS buffer:

Prepare 0.1M Tris, 0.15M NaCl, pH 7.4.

9.2 Blocking buffer (BB):

Prepare 3% BSA (w/v) in TBS.

9.3 1X Wash Buffer:

Dilute 50 mL of 10X wash buffer concentrate with 450 mL of deionized water.

10. Sample Preparation

- Use plasma (citrate) samples with this assay.
- Heparin or EDTA is not recommended. Heparin binds Factor X thus interfering with the assay.
- Centrifuge for 15 mins at 1000 *xg* within 30 mins of collection.
- Assay immediately or aliquot and store at ≤-20°C. Avoid repeated freezing and thawing.

11. Sample Dilution

- The assay measures Factor X antigen in the 2.5-500 ng/ml range.
- If the unknown is thought to have high Factor X levels, dilutions may be made in blocking buffer.
- A 1:1,000-1:2,000 dilution for normal mouse plasma or serum is suggested for best results.

12. Preparation of Standards

– Dilutions for the standard curve and zero standard must be made and applied to the plate immediately.

12.1 Reconstitute standard by adding 1 ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 1,000 ng/ml plasma standard.

12.2 Then dilute using Blocking buffer (BB) according to the table below:

Factor X concentration (ng/ml)	Dilutions
500	500µl (BB) + 500µl (from vial)
200	600µl (BB) + 400µl (500 ng/ml)
100	500µl (BB) + 500µl (200 ng/ml)
50	500µl (BB) + 500µl (100 ng/ml)
25	500µl (BB) + 500µl (50 ng/ml)
10	600µl (BB) + 400µl (20 ng/ml)
5	500µl (BB) + 500µl (10 ng/ml)
2.5	500µl (BB) + 500µl (5 ng/ml)
0	500µl (BB) Zero point to determine background

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- Vigorously shake plate (300 rpm) at each step of the assay.

13.1 Standard and Unknown Addition.

13.1.1 Remove microtiter plate from bag and add 100 µL Factor X standards (in duplicate) and unknowns to wells.

13.1.2 Carefully record position of standards and unknowns. Shake plate at 300 rpm for 30 mins.

13.1.3 Wash wells three times with 300 µL wash buffer. Remove excess wash by gently tapping plate on paper towel.
Δ Note: The assay measures Factor X antigen in the 2.5-500 ng/ml range. If the unknown is thought to have high Factor X levels, dilutions may be made in blocking buffer. A 1:1,000-1:2,000 dilution for normal mouse plasma or serum is suggested for best results.

13.2 Primary Antibody Addition.

13.2.1 Reconstitute primary antibody by adding 10 mL of blocking buffer directly to the vial and agitate gently to completely dissolve contents.

13.2.2 Add 100 µL to all wells. Shake plate at 300 rpm for 30 mins.

13.2.3 Wash wells three times with 300 µL wash buffer. Remove excess wash by gently tapping plate on paper towel.

13.3 Secondary Antibody Addition.

13.3.1 Briefly centrifuge vial before opening.

13.3.2 Dilute 2.5 µL of HRP conjugated streptavidin antibody into 2.5 mL blocking buffer diluent to generate a 1:1,000 dilution.

13.3.3 Add 0.2 ml of 1:1,000 dilution to 9.8 ml of diluent to generate a 1:50,000 dilution.

13.3.4 Add 100 µL the 1:50,000 dilution to all wells. Shake plate at 300 rpm for 30 mins.

13.3.5 Wash wells three times with 300 µL wash buffer. Remove excess wash by gently tapping plate on paper towel.

13.4 Substrate Incubation.

13.4.1 Add 100 µL TMB substrate to all wells and shake plate for 1 to 5 mins. Substrate will change from colorless to different strengths of blue.

13.4.2 Quench reaction by adding 50 µL of 1N H_2SO_4 or HCl 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 by gently shaking the plate.

13.5 Measurement.

13.5.1 Set the absorbance at 450 nm in a microtiter plate spectrophotometer.

13.5.2 Measure the absorbance in all wells at 450 nm.

13.5.3 Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

13.6 Calculation of Results.

13.6.1 Plot A_{450} against the amount of Factor X in the standards.

13.6.2 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.

13.6.3 Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit.

13.6.4 The amount of Factor X in the unknowns can be determined from this curve.

13.6.5 If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

14. Typical Results

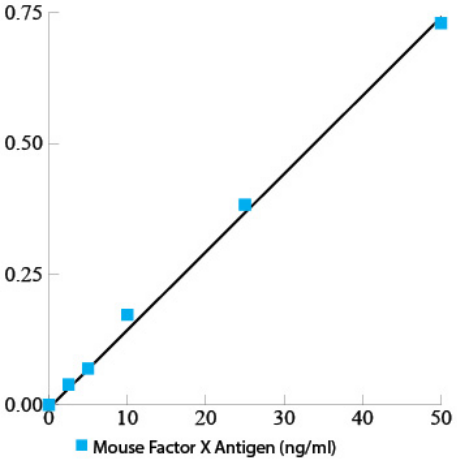

Fig 1. A typical standard curve. Example only.

15. Performance Characteristics

Expected Values:

The concentration of Factor X in normal human plasma was found to be 10 µg/ml. 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.

Sensitivity:

The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD450: 0.069-0.075) and calculating the corresponding concentration.

The MDD was 0.146 ng/ml.

Sample Values:

Samples were evaluated for the presence of the antigen.

Sample	Dilution	Mean (µg/ml)
Citrate Plasma	1:1,000	22.7
	1:2,000	23.1

16. Specificity

This assay recognizes natural and recombinant mouse Factor X and Factor Xa. Pooled normal plasma from cyno monkey, human, rat, dog, sheep, rabbit, and pig were assayed and no significant cross-reactivity was observed.

17. Notes

Technical Support

For all technical or commercial enquiries please go to:

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