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Total Human Coagulation Factor X ELISA Kit

Catalog No.: CSI20521A **Lot No.:** TBD **Size:** 1 plate (1 x 96 tests) **Expiration Date:** TBD
Catalog No.: CSI20521B **Lot No.:** TBD **Size:** 5 plates (5x 96 tests) **Expiration Date:** TBD

NOTE: this is a sample protocol which is subject to variation by Lot Number. Refer to the protocol inserted in your package for the current lot number specifications and expiration date or contact our technical support at tech@cellsciences.com

Specificity:	Natural and recombinant human Factor X and Factor Xa
Sensitivity:	0.060 ng/mL
Range:	0.058 – 0.070 ng/mL
Sample Type:	Human plasma

Background:

Factor X is a disulfide linked two-chain glycoprotein zymogen and is the precursor of the coagulation enzyme Factor Xa. Factor X is activated by Factor IXa in complex with Factor VIII, calcium and phospholipids during the intrinsic pathway and by Factor VIIa in complex with Tissue Factor, calcium and phospholipids during the extrinsic pathway of the coagulation cascade.

Assay Principle:

Human Factor X and Xa will bind to the capture antibody coated on the microtiter plate. After appropriate washing steps, polyclonal anti-human Factor X primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody, which is proportional to the total Factor X present in the samples, is reacted with the secondary antibody. Following an additional washing step, TMB substrate is used for color development at 450 nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of human Factor X. Color development is proportional to the concentration of Factor X in the samples.

Reagents Provided:

Item P. **96-well microtiter strip plate:** 8 x 12 removable well strips containing anti-human Factor X capture antibody dried and blocked on the surface.

Item A. **Human Factor X standard:** 1 vial of lyophilized standard

Item B. **10X Wash Buffer:** 1 bottle of 50 mL

Item C. **Anti-human Factor X primary antibody:** 1 vial of lyophilized polyclonal antibody

Item D. **TMB substrate solution:** 1 bottle of 10 ml solution

Item E. **Anti-rabbit horseradish peroxidase secondary antibody:** 1 vial of concentrated HRP labeled antibody

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 and primary antibody may be stored at -80°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 beyond the expiration date.

Reagents and Equipment Required:

- Microtiter plate shaker capable of 300 rpm uniform horizontally 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)

Precautions:

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.

- **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 kept refrigerated (4°C).
- **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 (w/v) in TBS

1x Wash buffer: Dilute 50 mL of the 10x Wash Buffer Concentrate with 450 mL of deionized water.

Sample Collection:

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 xg within 30 minutes of collection. Assay immediately or aliquot and store ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Assay Procedure:

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

Preparation of Standard:
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 100 ng/mL standard solution.

Dilution Table for preparation of Factor X Standard

Factor X Concentration (ng/ml)	Dilutions
50	500 µl (BB) + 500 µl (from vial)
20	600 µl (BB) + 400 µl (50 ng/ml)
10	500 µl (BB) + 500 µl (20 ng/ml)
5	500 µl (BB) + 500 µl (10 ng/ml)
2	600 µl (BB) + 400 µl (5 ng/ml)
1	500 µl (BB) + 500 µl (2 ng/ml)
0.5	500 µl (BB) + 500 µl (1 ng/ml)
0.2	600 µl (BB) + 400 µl (0.5 ng/ml)
0.1	500 µl (BB) + 500 µl (0.2 ng/ml)
0	500 µl (BB) Zero point to determine background.

NOTE: DILUTIONS FOR THE STANDARD CURVE MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition:
Remove microtiter plate from bag and add 100 µl Factor X standards (in duplicate) and unknowns to wells, be sure to 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 laboratory wipe.

NOTE: The assay measures Factor X in the 0.1 - 50 ng/mL range. If the unknown is thought to have high Factor X levels, dilutions may be made in blocking buffer. A 1:2000 – 1:8000 dilution for normal plasma is suggested for best results.

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 laboratory wipe.

Secondary Antibody Addition:

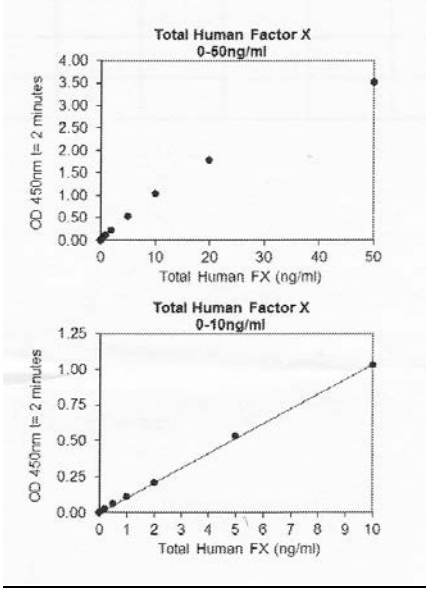
Briefly centrifuge vial prior to opening. Dilute 1 µL of conjugated secondary antibody in 10 mL of blocking buffer and add 100 µL to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µL wash buffer. Remove excess wash by gently tapping plate on paper towel or laboratory wipe.

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 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.

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}).

Calculation of Results:
Plot A_{450} against the amount of Factor X in the standards. 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. 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 Factor X in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

Typical Standard Curve (EXAMPLE ONLY, DO NOT USE)



Expected Values:

The concentration of Factor X in human plasma is 7-8 µg/mL.

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 (range OD_{450}: 0.058-0.070) and calculating the corresponding concentration. The MDD was 0.060 ng/mL.

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

Sample	1	2	3
n	20	20	20
Mean (ng/mL)	0.71	2.07	12.63
Standard Deviation	0.039	0.155	0.095
CV(%)	5.44	5.23	2.45

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	114	106	119	107
Range	101-125%	101-110%	112-124%	101-112%

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

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

Sample Type	Dilution	Mean (µg/mL)
Citrate Plasma	1:1,000	5.59
	1:2,000	6.08

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Example of ELISA Plate Layout
96 Well Plate: 20 Standard wells, 76 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml		
B	0	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml		
C												
D												
E												
F												
G												
H												

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