

### INTENDED USE

This human coagulation Factor X antigen assay is intended for the quantitative determination of total Factor X antigen in human plasma. **For research use only.**

### BACKGROUND

Factor X is a disulfide linked two-chain glycoprotein zymogen and is the precursor of the coagulation enzyme Factor Xa [1]. 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 [2].

### ASSAY PRINCIPLE

Human Factor X will bind to the capture antibody coated on the microtiter plate. Factor X and Xa will react with the antibody on the 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 450nm. 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.

### STANDARD CALIBRATION

Factor X standard provided is calibrated against the WHO 4th International Standard for Factor X, Plasma, Human distributed by NIBSC (09/172), South Mimms, Potters Bar, Hertfordshire, UK.

Lot 1117L: 100 ng = 0.0146 IU

### REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-human Factor X antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Human Factor X standard:** 1 vial lyophilized standard
- **Anti-human Factor X primary antibody:** 1 vial lyophilized polyclonal antibody
- **Anti-rabbit horseradish peroxidase secondary antibody:** 1 vial concentrated HRP labeled antibody
- **TMB substrate solution:** 1 bottle of 10ml solution

### STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standard and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

### OTHER REAGENTS AND SUPPLIES 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<sub>2</sub>SO<sub>4</sub> or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

## PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- 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.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- 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 (w/v) in TBS
- 1X Wash buffer: Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water

## SAMPLE COLLECTION

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at  $\leq -20^{\circ}\text{C}$ . 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 1ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 100ng/ml standard solution.

Dilution table for preparation of Factor X standard:

Factor X concentration (ng/ml)	Dilutions
50	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (from vial)
20	600 $\mu\text{l}$ (BB) + 400 $\mu\text{l}$ (50ng/ml)
10	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (20ng/ml)
5	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (10ng/ml)
2	600 $\mu\text{l}$ (BB) + 400 $\mu\text{l}$ (5ng/ml)
1	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (2ng/ml)
0.5	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (1ng/ml)
0.2	600 $\mu\text{l}$ (BB) + 400 $\mu\text{l}$ (0.5ng/ml)
0.1	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (0.2ng/ml)
0	500 $\mu\text{l}$ (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 and add 100 $\mu\text{l}$  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 300 $\mu\text{l}$  wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures Factor X antigen 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 1:2,000-1:8,000 dilution for normal plasma is suggested for best results.

### Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100 $\mu\text{l}$  to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 $\mu\text{l}$  wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

### Secondary Antibody Addition

Briefly centrifuge vial before opening. Dilute 2 $\mu\text{l}$  of conjugated secondary antibody in 10ml of blocking buffer and add 100 $\mu\text{l}$  to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 $\mu\text{l}$  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<sub>2</sub>SO<sub>4</sub> 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.

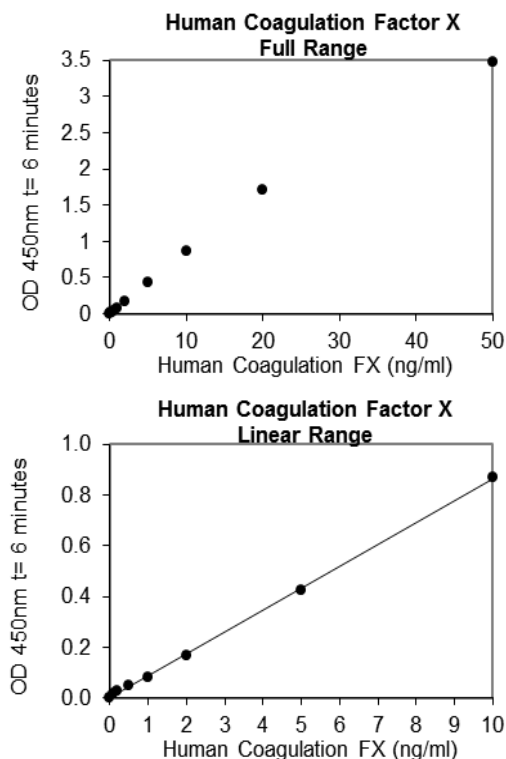
### Measurement

Set the absorbance at 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<sub>450</sub>).

### Calculation of Results

Plot A<sub>450</sub> 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.

A typical standard curve (EXAMPLE ONLY):



### EXPECTED VALUES

The concentration of Factor X in human plasma is 7-8 µg/ml [3].

### 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<sub>450</sub>: 0.086-0.095) and calculating the corresponding concentration. The MDD was 0.094 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	2.97	12.63
Standard Deviation	0.039	0.155	0.095
CV (%)	5.44	5.23	2.45

**Inter-assay Precision:** These studies are currently in progress. Please contact us for more information.

**Recovery:** The recovery of antigen spiked to levels throughout the range of the assay in blocking buffer was evaluated.

Sample	1	2	3	4
n	4	4	4	4
Mean (ng/ml)	0.73	3.08	13.2	33.9
Average % Recovery	98	103	88	113
Range	85-107%	98-109%	84-91%	102-120%

**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 ( $\mu\text{g/mL}$ )
Citrate Plasma	1:1,000	5.59
	1:2,000	6.08

**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												

**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 of or contact with the above product.

**REFERENCES**

1. Jackson CM: Biochemistry. 1972, 11:4873.
2. Fujikawa K, *et al.*: Biochemistry. 1974, 13:5290.
3. Miletich JP, *et al.*: Methods in Enzymology. 1981, 80:221.

SAMPLE INSERT  
 Refer to kit box for  
 lot specific instructions