Catalog # HFXKT-TOT

Total Human Coagulation Factor X Antigen Assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

This human coagulation Factor X antigen assay is intended for the quantitative determination of total Factor X antigen in human plasma.

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.

REAGENTS PROVIDED

- **96-well microtiter strip plate:**
  8X12 removable well strips containing anti-human Factor X capture antibody dried and blocked on the surface
- **10X Wash Buffer:**
  1 bottle of 50ml; bring to 1X using DI water
- **Human Factor X standard:**
  1 vial of lyophilized standard
- **Anti-human Factor X primary antibody:**
  1 vial of lyophilized polyclonal antibody
- **Anti-rabbit secondary antibody:**
  1 vial concentrated HRP labeled antibody
- **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

- 1-channel pipettes covering 0-10µl and 200-1000µl
- 12-channel pipette covering 30-300µl
- Paper towels or kimwipes
- 50ml tubes, 1.5ml centrifuge tubes
- 1N H_2SO_4
- DI water

- Magnetic stirrer and stir-bars
- Plastic containers with lids
- Microtiter plate spectrophotometer operable at 450nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300rpm. (OPTIONAL)

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.
- **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 in TBS
- **Wash buffer concentrate:** The wash buffer supplied in a 10X concentrate and must be diluted 1:10 with deionized water for use with the kit.

SPECIMEN COLLECTION

The assay measures total human Factor X in the 0.1-50 ng/ml range. Samples giving human Factor X levels above 100ng/ml should be diluted in blocking buffer before use. A 1:1,000 dilution for plasma is suggested for best results.

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 the vial to give a 100ng/ml solution.

Dilution table for preparation of human Factor X standards:

Factor X concentration (ng/ml)	Dilutions
50	300µl (BB) + 300µl (100ng/ml)
20	300µl (BB) + 200µl (50ng/ml)
10	250µl (BB) + 250µl (20ng/ml)
5	250µl (BB) + 250µl (10ng/ml)
2	300µl (BB) + 200µl (5ng/ml)
1	250µl (BB) + 250µl (2ng/ml)
0.5	250µl (BB) + 250µl (1ng/ml)
0.2	300µl (BB) + 200µl (0.5ng/ml)
0.1	250µl (BB) + 250µl (0.2ng/ml)
0	500µl (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. Add 100µl 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:
Add 10ml of blocking buffer directly to the primary antibody 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.

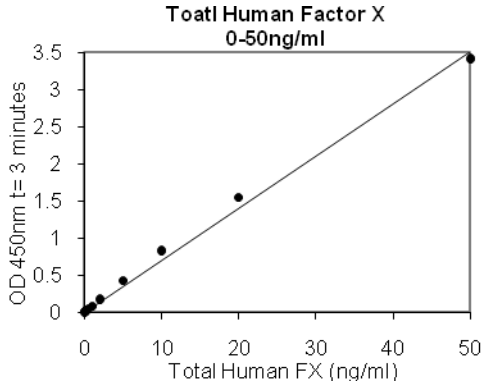
Secondary Antibody Addition:
Dilute 1µl into 10ml BB and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

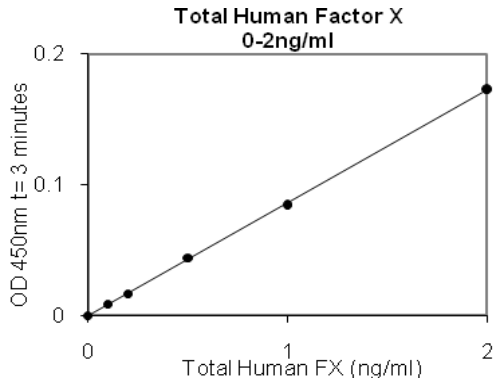
Substrate Incubation:
Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 stop solution to all wells when samples are visually in the same range as the standards. Add stop 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 human Factor X in the standards. Fit a straight line through the points using a linear fit procedure. The amount of total human 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 Factor X in human plasma is 7-8 µg/ml [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.

REFERENCE

1. Jackson CM. Characterization of Two Glycoprotein Variants of Bovine Factor X and Demonstration That the Factor X Zymogen Contains Two Polypeptide Chains. Biochemistry 1972; 11:4873.

2. Fujikawa K *et al*. The Mechanism of Activation of Bovine Factor X (Stuart Factor) by Intrinsic and Extrinsic Pathways. Biochemistry 1974; 13:5290.

3. Miletich JP *et al*. Purification of Human Coagulation Factors II, IX, and X Using Sulfated Dextran Beads. Methods in Enzymology 1981; 80:221.

Example of ELISA Plate Layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.1ng/ml	0.2ng/ml	0.5ng/ml	1.0ng/ml	2.0ng/ml	5.0ng/ml	10ng/ml	20ng/ml	50ng/ml		
B	0	0.1ng/ml	0.2ng/ml	0.5ng/ml	1.0ng/ml	2.0ng/ml	5.0ng/ml	10ng/ml	20ng/ml	50ng/ml		
C												
D												
E												
F												
G												
H												

96 Well Plate
Standards: 20 Wells
Samples: 76 Wells