Catalog # HFXIKT-TOT

Total Human Coagulation Factor XI Antigen Assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

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

BACKGROUND

Factor XI is a disulfide linked two-chain glycoprotein zymogen and is the precursor of the coagulation enzyme Factor XIa [1]. Factor XI consists of two identical monomers and circulates in plasma in complex with kininogen [2]. Factor XI is activated by Factor XIIa and converts Factor IX to Factor IXa during the intrinsic pathway of the coagulation cascade [3].

ASSAY PRINCIPLE

Human Factor XI will bind to the affinity purified capture antibody coated on the microtiter plate. Factor XI and XIa will react with the antibody on the plate. After appropriate washing steps, peroxidase labeled polyclonal anti-human Factor XI primary antibody binds to the captured protein. Excess antibody is washed away and 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 XI. Color development is proportional to the concentration of Factor XI in the samples.

STANDARD CALIBRATION

Factor XI standard provided is calibrated against the WHO 1st International Standard for Factor XI, Plasma, Human distributed by NIBSC (04/102), South Mimms, Potters Bar, Hertfordshire, UK.

Lot 911L: 1000 ng = 0.303 IU

REAGENTS PROVIDED

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

WARNINGS

Warning – Avoid skin and eye contact when using TMB 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 TMB substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the TMB 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 XI in the 0.2-100 ng/ml range. Samples giving human Factor XI 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 1,000ng/ml solution.

Dilution table for preparation of human Factor XI standards:

Factor XI concentration (ng/ml)	Dilutions
100	900µl (BB) + 100µl (1000ng/ml)
50	500µl (BB) + 500µl (100ng/ml)
20	600µl (BB) + 400µl (50ng/ml)
10	500µl (BB) + 500µl (20ng/ml)
5	500µl (BB) + 500µl (10ng/ml)
2	600µl (BB) + 400µl (5ng/ml)
1	500µl (BB) + 500µl (2ng/ml)
0.5	500µl (BB) + 500µl (1ng/ml)
0.2	600µl (BB) + 400µl (0.5ng/ml)
0	500µl (BSA) 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.

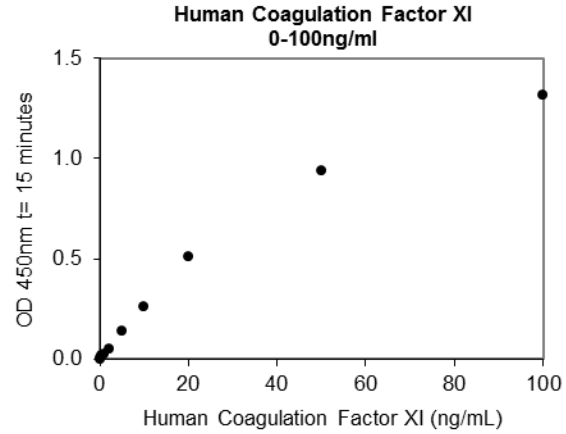
Primary Antibody Addition:
Add 10ml of blocking buffer directly to the primary antibody vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

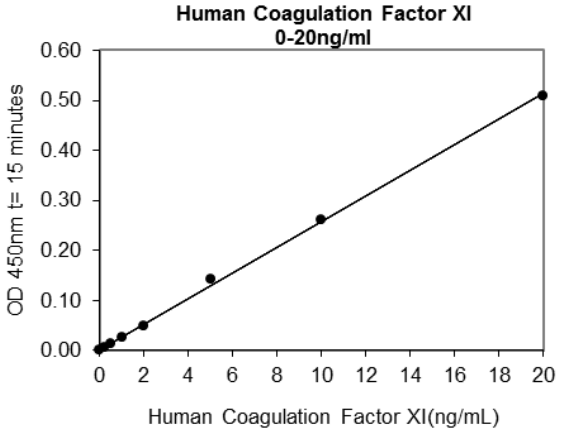
Substrate Incubation:
Add 100µl TMB substrate to all wells and shake plate for 15-20 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 stop solution to all wells when samples are visually in the same range as the standards. Add 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 XI in the standards. Fit a straight line through the points using a linear fit procedure. The amount of total human Factor XI in the unknowns can be determined from this curve.

A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)





EXPECTED VALUES

The concentration of Factor XI in normal human plasma ranges from 3.0 to 6.0 µg/ml [4].

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. Kurachi K and EW Davie. Human Factor XI (Plasma Thromboplastin Antecedent). Methods in Enzymology 1981; 80:211-220.
2. Thompson RE *et al*. Association of factor XI and high molecular weight kininogen in human plasma. J. Clin. Invest. 1977; 60:1376.
3. Walsh PN *et al*. Factor XI. Methods in Enzymology 1993; 222:65-96.
4. Bouma BN *et al.* Immunologic studies of human coagulation factor XI and its complex with high molecular weight kininogen. Blood 1983; 62:1123-1131.

Example of ELISA Kit Plate Layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.2ng/ml	0.5ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100ng/ml		
B	0	0.2ng/ml	0.5ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100ng/ml		
C												
D												
E												
F												
G												
H												

96 Well Plate
Standards: 20 Wells
Samples: 76 Wells