Total Human Coagulation Factor XI ELISA

For Research Use Only

INTRODUCTION

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

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].

PRINCIPLES OF PROCEDURE

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.

MATERIALS PROVIDED

Component	Contents	Quantity	Storage	Cat. No.
Coated Plate	Anti-human Factor XI coated 96-well plate	1 plate	4°C	CF46a
Standard	Lyophilized Human Factor XI standard	1 vial	4°C	CF46b
Wash Buffer	10x solution for washing plate	50 mL	4°C	CF46c
Primary Antibody	Lyophilized Anti-human Factor XI-HRP Conjugate	1 vial	4°C	CF46d
Substrate	TMB Substrate	10 mL	4°C	CF46e

MATERIALS NEEDED BUT NOT PROVIDED

1. Pipettes covering 0-10 μl and 200-1000 μl and tips
2. 12-channel pipette covering 30-300 μl
3. 1 N H_2SO_4
4. DI water
5. Microtiter plate spectrophotometer with a 450 nm filter
6. Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm

STORAGE CONDITIONS

1. Store this kit and its components at 4°C until use.
2. The reconstituted Standard and Primary Antibody may be stored at -70°C for later use. **DO NOT** freeze/thaw the Standard or Primary Antibody more than once.

PROCEDURAL NOTES

1. Use aseptic technique when opening and dispensing reagents.
2. This kit is designed to work properly as provided and instructed. Additions, deletions, or substitutions to the procedure or reagents are not recommended, as they may be detrimental to the assay.
3. Exercise universal precautions during the performance or handling of this kit or any component contained therein.

SAMPLE COLLECTION AND PREPARATION

The assay measures total human Factor XI in the 0.1-100 ng/ml range. Samples giving human Factor XI levels above 100 ng/ml should be diluted in Blocking Buffer before use. A 1:1,000 to 1:5,000 dilution for plasma is suggested for best results.

REAGENT PREPARATION

1. **10x Wash Buffer:** Dilute the 50 mL of concentrate to 1x with 450 mL of DI water prior to use.
2. **TBS Buffer:** 0.1 M Tris, 0.15 M NaCl, pH 7.4.
3. **3% BSA Blocking Buffer:** 3% BSA in TBS Buffer.
4. **Standard:** Reconstitute with DI Water as directed on the vial to give a 1000 ng/mL Standard Stock Solution. Prepare immediately prior to use.
5. **Primary Antibody:** Reconstitute with 3% BSA Blocking Buffer as directed on the vial and vortex gently to mix. Prepare immediately prior to use.

SANDARD PREPARATION

Prepare the Standard Stock Solution as described above. **Do not prepare the standards until you are ready to apply them to the plate.**

Table 1: Preparation of Standard Curve

Standard	Factor XI Concentration (ng/mL)	Blocking Buffer (μL)	Transfer Volume (μL)	Transfer Source	Final Volume (μL)
S_9	100	900	100	Stock Vial	500
S_8	50	500	500	S_9	600
S_7	20	600	400	S_8	500
S_6	10	500	500	S_7	500
S_5	5	500	500	S_6	600
S_4	2	600	400	S_5	500
S_3	1	500	500	S_4	500
S_2	0.5	500	500	S_3	600
S_1	0.2	600	400	S_2	1000
S_0	0	500	---	---	500

ASSAY PROCEDURE

1. Add 100 μl of the Standards and unknowns to the wells in duplicate. Shake the plate at 300 rpm for 30 minutes at room temperature (RT). See Scheme I for a suggested plate layout.
2. Wash the plate 3 times according to the following wash procedure:
   a. Remove the contents of each well by inversion of the plate.
   b. Tap out the remaining contents of the plate onto a lint free paper towel.
   c. Add 300 μL of 1x Wash Buffer.
   d. Let stand for 2-3 minutes.
   e. Repeat procedure two more times, then proceed to step "f".
   f. Remove the contents of each well by inversion of plate into an appropriate disposal device.
   g. Tap out the remaining contents of the plate onto a lint free paper towel, then proceed to step 3.
3. Add 100 μl of the Primary Antibody to each well. Shake the plate at 300 rpm for 30 minutes at RT.
4. Wash the plate three times as in step 2.
5. Add 100 μl of TMB Substrate to each well. Shake the plate at 300 rpm for 5-10 minutes at RT.
6. Stop the reaction by adding 50 μl of 1N H_2SO_4 to each well and read the plate at 450 nm.

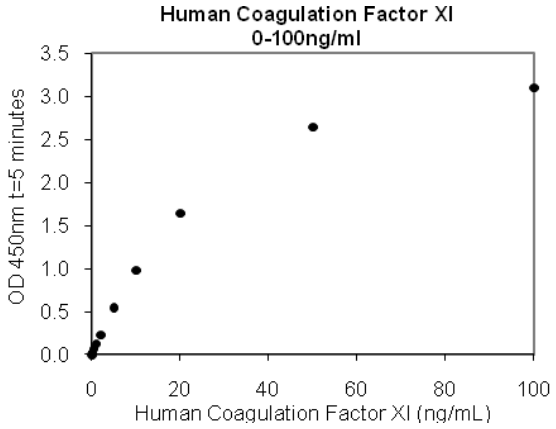
Scheme I: Suggested Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S_9	S_8	S_7	S_6	S_5	S_4	S_3	S_2	S_1	S_0	U_1	U_2
B	S_9	S_8	S_7	S_6	S_5	S_4	S_3	S_2	S_1	S_0	U_1	U_2
C	U_3	U_4	U_5	U_6	U_7	U_8	U_9	U_{10}	U_{11}	U_{12}	U_{13}	U_{14}
D	U_3	U_4	U_5	U_6	U_7	U_8	U_9	U_{10}	U_{11}	U_{12}	U_{13}	U_{14}
E	U_{15}	U_{16}	U_{17}	U_{18}	U_{19}	U_{20}	U_{21}	U_{22}	U_{23}	U_{24}	U_{25}	U_{26}
F	U_{15}	U_{16}	U_{17}	U_{18}	U_{19}	U_{20}	U_{21}	U_{22}	U_{23}	U_{24}	U_{25}	U_{26}
G	U_{27}	U_{28}	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	U_{37}	U_{38}
H	U_{27}	U_{28}	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	U_{37}	U_{38}

CALCULATIONS

1. Plot the A_{450} against the concentration of Factor XI in the standards.
2. Fit a straight line through the points using a linear fit procedure.
3. Calculate the Factor XI concentrations in the unknowns using the equation generated by the standard curve.

Figure 1: Typical Standard Curve



EXPECTED VALUES

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

REFERENCES

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2. Thompson, R.E., *et al.*; (1977) J. Clin, Invest.: **60**: 1376
3. Walsh, P.N,; (1993) Methods in Enzymology: **222**:65-96
4. Bouma, B.N., *et al.*; (1983) Blood: **62**:1123-1131

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