Affinity Biologicals™ INC.

REPRESENTATIVE DATASHEET*

VisuLize™ FXI Antigen Kit

96 Test Enzyme Immunoassay Kit for
Factor XI (FXI) antigen

For Research Use Only.
Not for use in diagnostic procedures.

Product # FXI-AG

Store at 2–8°C. Do not freeze.

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INTENDED USE

The *VisuLize*™ FXI Antigen kit is an Enzyme Immunoassay for the quantitative determination of Factor XI antigen in human plasma samples using the double antibody enzyme linked immuno-sorbent assay (ELISA).

SUMMARY

Factor XI (FXI) is a coagulation protein produced in the liver that circulates in plasma at approximately 5 µg/ml (30 nM). The mass of FXI is 160 kDa as determined by SDS-PAGE under non-reducing conditions and 80 kDa upon reduction. FXI consists of two identical 80 kDa subunits linked by disulphide bonds. Each subunit consists of a tandem repeat of four apple domains followed by a serine protease catalytic domain. Cleavage of FXI by activated factor XII or thrombin converts each subunit into a two-chain form and generates two active sites per molecule. The mass of FXIa is 160 kDa unreduced, but upon reduction FXIa migrates as a heavy chain of 50 kDa and a light chain of 30 kDa. The catalytic site of FXIa resides in the light chain. In plasma, FXI or FXIa circulates in non-covalent 1:1 complex with high molecular weight kininogen, which acts as a cofactor in the activation of FXI by activated factor XII. The activity of FXIa is regulated by platelets and by several proteinase inhibitors including, in order of decreasing importance, C1-inhibitor, $\alpha_2$antiplasmin, $\alpha_1$antitrypsin and antithrombin. Heparin has relatively little effect on the rate of inhibition of FXIa by antithrombin. The only known natural substrate for activated FXI (FXIa) is factor IX (Christmas factor) and the only cofactor required for this reaction is ionized calcium[1-3].

PRINCIPLE OF ENZYME IMMUNOASSAY

Strip wells are pre-coated with goat polyclonal antibody to human FXI. Plasma samples are diluted and applied to the wells. The FXI antigen present binds to the coated antibody. After washing away unbound material, peroxidase-labeled goat detecting antibody is applied and allowed to bind to the captured FXI. The wells are again washed and a solution of TMB (the peroxidase substrate tetramethylbenzidine) is applied and allowed to react for a fixed period of time. A blue color develops which changes to yellow upon quenching the reaction with acid. The color formed is measured spectrophotometrically in a microplate reader at 450 nm. The absorbance at 450 nm is directly proportional to the quantity of FXI antigen captured onto the well. The assay is calibrated using the calibrator plasma provided in the kit.

REAGENTS

A. Description of Provided Items

Item 1: Foil pouch containing 6 strips, each containing 16 wells coated with goat antibody to human FXI
Item 2: 2 vials of Calibrator Plasma, each lyophilized from 1 mL
Item 3: 2 vials of Control Plasma A, each lyophilized from 1 mL
Item 4: 2 vials of Control Plasma B, each lyophilized from 1 mL
Item 5: 1 vial containing 50 mL of 20X Wash Buffer Concentrate
Item 6: 3 vials, each containing 20 mL of 2X buffered Sample Diluent
Item 7: 1 vial containing 12 mL peroxidase-labeled goat detecting antibody
Item 8: 1 vial containing 12 mL of TMB substrate
Item 9: 1 vial containing 12 mL Stop Solution (0.2 M Sulphuric acid)

B. Caution and Warning

For Research Use Only. Not for use in diagnostic procedures.
This kit is intended for use by personnel trained in laboratory procedures and universal precautions for the use of chemicals and potentially biohazardous substances. Some items contain human source material. Although this material was prepared with plasma collected from donors screened for CJD and was tested at source and found negative for HBsAg, syphilis and antibodies to HIV and HCV and non-reactive for HIV-1 rNA and HCV rNA by FDA approved tests, it should be handled by personnel trained in the proper procedures for handling potential viral contaminants as no test can offer complete assurance that products derived from human blood will not transmit infectious diseases. As with all materials of human origin, this product should be handled as a potentially infectious material.
The substrate TMB (tetramethylbenzidine) has reduced toxicity, but precautions should still be taken to avoid direct contact. The use of gloves and safety glasses is recommended.
The Stop Solution contains dilute sulphuric acid (0.2 M), which is corrosive. The use of gloves and safety glasses is recommended.
The disposal of waste materials must be carried out according to current local regulations.
For a Material Safety Data Sheet for this product, contact Affinity Biologicals Inc.

C. Reagent Preparation

Item 1 (Antibody-coated strips with frame): Just prior to use, open pouch and remove strips and frame. Unused strips should be replaced in the pouch and resealed. Strips may be used directly, see Procedure section C: Assay Procedure.
Item 2 (Calibrator plasma): Reconstitute one vial with 1.0 mL of reagent grade water. Allow contents to dissolve for 15 minutes at room temperature with occasional swirling. Stability after reconstitution is 4 hours at 2-8°C or ambient (18-25°C), or 30 days at –20° C.
Items 3 and 4 (Control plasmas): Reconstitute one vial of each plasma with 1.0 mL of reagent grade water. Allow contents to dissolve for 15 minutes at room temperature with occasional swirling. Stability after reconstitution is 4 hours at 2-8°C or ambient (18-25°C), or 30 days at –20° C.
Item 5 (20X Wash Buffer Concentrate): Allow vial to warm to room temperature before use. Ensure any crystals that may have formed are dissolved before proceeding. If necessary the vial can be warmed to 37°C until all crystals have dissolved. Dilute the concentrate 1/20 before use. For every 2 strips (32 wells), add 16 mL concentrate to 304 mL reagent grade water and mix. Stability after dilution is 1 week at 2–8°C.

Item 6 (2X Sample Diluent Concentrate): Allow vial to warm to room temperature before use. Ensure any crystals that may have formed are dissolved. If necessary the vial can be warmed to 37°C until all crystals have dissolved. Dilute the concentrate by adding volume of concentrate to an equal volume of reagent grade water and mix. Stability after dilution is 1 week at 2–8°C.

Items 7-9 are supplied ready to use.

D. Storage and Stability

Intact kits and un-reconstituted reagents are stable until the expiration date stated on the box and individual reagent labels when stored at 2–8°C.

SPECIMEN COLLECTION

Blood is collected into 3.2% Buffered Citrate anticoagulant tubes at a ratio of 9 volumes blood to 1 volume anticoagulant and gently mixed by inversion. Centrifuge at a minimum of 1500 x g for 15 minutes (CLSI Guideline H21-A5[4]). Remove supernatant plasma and use within 4 hours or freeze below –20°C for up to 1 month.

PROCEDURE

A. Material Provided

Foil pouch containing 6 strips of antibody coated wells
Calibrator Plasma, lyophilized
Control Plasma A, lyophilized
Control Plasma B, lyophilized
20X Wash Buffer Concentrate
2X Sample Diluent Concentrate
Detecting antibody solution
TMB substrate
Stop Solution
Adhesive Plate Sealer

B. Additional Material Required (but not provided)

Reagent grade water for reconstitution and dilution of reagents
Single-channel adjustable volume pipettes
Multi-channel pipettes
Pipette Tips
Laboratory timer
Microplate strip-well washer device
Microplate compatible spectrophotometer capable of 450 nm.

C. Assay Procedure

PROCEDURAL NOTES:

- Reconstitute reagents as described in REAGENTS, Section C, Reagent Preparation. Allow reagents to warm to room temperature before use.
- It is recommended that all calibrator, control and test sample dilutions be run in duplicate and that each run include a buffer blank (see Assay Calibration section).
- All dilutions must be made just prior to use in the assay.
- Do not allow the wells to become dry at any time. Keep plate covered during incubations.
- Plasma samples should not be applied at dilutions lower than 1/5.
- Do not use kit components from different lot numbers.
- Incubation temperatures above or below normal room temperature (18 -25°C) may contribute to inaccurate results.
- Do not use kit components beyond expiration date
- Used strips must be discarded and not re-used.

1. **Preparation of Calibrator Plasma Dilutions:** Dilute the Calibrator Plasma (reconstituted Item 2) into sample diluent (diluted Item 6) as detailed in Table 1 below:

TABLE 1:

Dilution	Calibrator Plasma	Sample Diluent
100% **	20 µL	980 µL
50%	350 µL of 100%	350 µL
25%	350 µL of 50%	350 µL
12.5%	350 µL of 25%	350 µL
6.25%	350 µL of 12.5%	350 µL
3.13%	350 µL of 12.5%	350 µL

(Note: 100% = 1.0 IU/ml)

** Refer to Calibrator Plasma vial (Item 2) for FXI antigen value to be used as the concentration of the initial dilution of the calibrator plasma. E.g. If the calibrator has an assigned value of 1.25 IU/ml, follow the same dilution scheme above but call the first point of the calibration curve 1.25 IU/ml.

2. **Control plasma A** (reconstituted Item 3) and **normal test plasmas** are diluted 1/100 and 1/200. Add 10 µL plasma into 990 µL diluted sample diluent, mix, then add 350 µL of this 1/100 dilution into 350 µL diluted sample diluent to obtain the 1/200 dilution. **Control Plasma B** (reconstituted Item 4) and samples low in FXI antigen should be run at lower dilutions of 1/25 and 1/50. Add 40 µL plasma into 960 µL sample diluent (Item 6), mix, then add 350 µL of this 1/25 dilution into 350 µL sample diluent to obtain the 1/50 dilution. For samples with expected FXI antigen levels of <5%, dilute 1/5 i.e. 60 µL plasma into 240 µL diluted sample diluent.

3. **Assay**

PLATE PREPARATION	Place desired number of strips into frame.	
STEP	Pipette into each pre-coated well:	
FXI CAPTURE	Test Sample (run in duplicate)	100 µL
	Cover strips with the plate sealer and incubate 1 hour at ambient temperature.	
Empty wells and wash with 300 µl diluted wash buffer 3 times.		
DETECTING ANTIBODY	Detecting Antibody Solution (Item 7)	100 µL
	Cover strips with the plate sealer and incubate 1 hour at ambient temperature.	
Empty wells and wash with 300 µl diluted wash buffer 3 times.		
COLOR DEVELOPMENT	TMB Substrate (Item 8)	100 µL
	Allow color to develop for **exactly** 10 minutes at ambient temperature.	
	Stop Solution (Item 9)	100 µL (Add to each well in same order in which the TMB was added)
Read plate at a wavelength of 450 nm within 30 minutes of adding Stop Solution. If necessary, keep plate frame for use with any unused strips. Discard used strips.		

CALIBRATION

A. Assay Calibration

The FXI antigen value stated on the Calibrator Plasma vial has been determined by comparison to the ISTH/SSC secondary coagulation standard for FXI activity. This FXI antigen value should be used as the concentration of the initial dilution of the calibrator plasma (i.e. the 100% calibrator dilution). It is recommended that the plate be blanked on wells that have received Sample Diluent alone instead of diluted sample (reagent blank wells).

B. Reference Curve and Calculation of Results

The reference curve is a log-log plot of the mean absorbance values (y axis) versus the FXI antigen concentration (x axis). The FXI antigen content of test samples and controls can be read from the reference curve and multiplied by the appropriate dilution factor. Under the conditions described here, a sample diluted 1/50 will have a dilution factor of 1, a dilution of 1/100 will have a dilution factor of 2, a dilution of 1/200 has a dilution factor of 4.

QUALITY CONTROL

The supplied Control Plasmas (Item 3 and 4) should be assayed with every series of samples that are run. The FXI antigen values obtained for test samples should be considered suspect if the values obtained for the control plasmas fall outside of the range stated on the Control Plasma labels.

LIMITATIONS AND INTERFERENCES

This kit has been developed for use with citrated plasma. The use of samples containing anticoagulants other than 3.2% sodium citrate is not recommended. Assay interference due to the presence of drugs or Rheumatoid Factor in test samples has not been reported. However, the potential for interference by high levels of heterophilic antibodies cannot be excluded. The theoretical possibility of test samples containing antibodies to goat immunoglobulin may also interfere in the assay.

EXPECTED VALUES

Each laboratory should determine a normal range independently but results from three lots measured in 72 healthy individuals indicate a normal reference interval for FXI antigen of 0.69-1.46 IU/mL (mean 1.07 IU/mL, SD = 0.193).

PERFORMANCE CHARACTERISTICS

A. Specificity

This assay measures Factor XI antigen in human plasma.

B. Detection Limit

When assay is performed as indicated in Section C, Assay Procedure, the detection limit of this assay is <0.01 IU/mL (<1 %) FXI antigen. The upper limit of detection may vary with each lot of kit depending on the assayed value of the calibrator plasma supplied in the kit. Samples with values outside the range of the reference curve should be re-tested at an appropriate dilution to obtain accurate results.

C. Precision

Within-run (intra-assay), between day (inter-assay), between-run and within device precision were assessed for three lots of the VisuLize FXI Antigen Kit using 3 levels of test plasmas. Plasma samples were tested in duplicate, 2 times per day for 10 days for a total of 20 assay events[5]. The coefficients of variation (% CV) obtained in these precision studies are presented in the table below.

	Within Run	Between Day	Between Run	Within Device Precision
Normal FXI Sample	3.6 - 6.7%	0 - 2.9%	1.2 – 4.3%	4.9 – 7.4%
Mid-level FXI Sample	3.8 – 7.0%	0 - 3.2%	0.7 – 4.4%	4.5 – 7.8%
Low FXI Sample	3.5 – 4.0%	0 – 1.7%	2.7 – 4.1%	4.9 – 5.4%

D. Lot-to-Lot Variability

90 samples with Factor XI antigen values ranging from 0.05–1.72 IU/mL were tested in duplicate on three lots to determine assay precision between lots. The mean lot-to-lot variability was 7.06%.

REFERENCES

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