



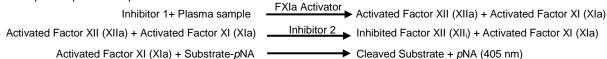
Factor XIa Activity Assay Kit (Colorimetric)

07/16

(Catalog # K973-100; 100 assays, Store kit at -20°C)

I. Introduction:

Factor XI or plasma thromboplastin antecedent (EC 3.4.21.27), the zymogen form of factor XIa, is a serine protease. In humans, Factor XI is encoded by the F11 gene. Factor XI (FXI) circulates as a homo-dimer in its inactive form activated into factor XIa by factor XIIa (FXIIa) via the "contact pathway", thrombin, and self-activation by its active form (FXIa). The deficiency of factor XI causes the rare hemophilia C which is an autosomal recessive disorder characterized by lower spontaneous bleeding, but excessive blood loss during surgical procedures. Low levels of factor XI also occur in many other disease states, including Noonan syndrome. High levels of factor XI have been implicated in thrombosis. Factor XIa also activates factor IX which, in turn, activates factor X in the coagulation cascade. BioVision's Factor XIa activity assay kit utilizes the ability of factor XIa to cleave a synthetic substrate to release p-Nitroaniline (pNA) which can be quantitatively measured by a colorimetric assay (OD 405 nm). The kit is easy-to-use and can detect Factor XIa (as low as 1 mPEU) from plasma and purified protein samples.



II. Applications:

Detection of enzymatic activities of factor XIa in plasma and purified protein samples

III. Sample Type:

· Plasma and purified protein samples

IV. Kit Contents:

Components	K973-100	Cap Code	Part Number
FXIa Assay Buffer	25 ml	WM	K973-100-1
FXIa Activator	1 ml	Clear	K973-100-2
Inhibitor 1	0.1 ml	Blue	K973-100-3
Inhibitor 2	0.1 ml	Orange	K973-100-4
FXIa Substrate	1 ml	Red	K973-100-5
Human Factor XIa	1 Vial	Green	K973-100-6
pNA Standard (0.1 M)	20 µl	Yellow	K973-100-7

V. User Supplied Reagents and Equipment:

- 96-well clear well plate
- Multi-well spectrophotometer
- Chloroform
- Plasma

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

- FXIa Assay Buffer: Bring to room temperature before use. Store at 4°C or -20°C.
- FXIa Activator: Bring to room temperature before use. After first use, this suspension can be stored at room temperature. Before each use, mix well.
- Inhibitor 1 and 2 and FXIa Substrate: Aliquot and store at -20°C. Avoid multiple freeze/thaw. Thaw on ice before use.
- Human Factor XIa: Reconstitute with 20 μl of FXIa Assay Buffer. Store at -20°C. Avoid repeated freeze/thaw. Use within two months.

VII. FXIa Activity Assay Protocol:

- 1. Sample Preparation: The following pretreatment of plasma with chloroform is recommended but not mandatory.
 - a) Chloroform Pretreatment: Take 50 µl of plasma in an Eppendorf tube and add 50 µl of cold chloroform. Mix well by inverting the tube for 1 min. Centrifuge the tube at 3000 x g for 5 min to separate two layers. Carefully pipette top layer containing pretreated plasma in a separate Eppendorf tube.
 - b) Use 1-10 μ I of the chloroform treated plasma sample in an Eppendorf tube and add 1 μ I of Inhibitor 1. Incubate at room temperature (RT) for 10 min. Add 10 μ I of Activator solution, mix well by gentle tapping the tube. Incubate at RT for additional 30-45 min.

Optional: Centrifuge the tube at 3000 x g for 5 min and remove the solution from activator.

- c) To the solution, add 1 µl of Inhibitor 2. Incubate further for 10 min at RT. Load this solution on a microplate well.
- d) As a negative control, a sample containing same volume of plasma without activator (Sample Background) can be run. For FXIa Positive Control, use 2-5 µl of reconstituted FXIa enzyme solution. Bring the final volume in each well to 50 µl with FXIa Assay Buffer.
- 2. pNA Standard: Dilute 5 μl 0.1 M pNA Standard into 95 μl FXla Assay Buffer to prepare 5 mM pNA. Add 0, 2, 4, 6, 8, 10 μl of 5 mM pNA standard into each well. Adjust volume to 100 μl/well with FXla Assay Buffer to generate 0, 10, 20, 30, 40, 50 nmol/well of pNA standard.

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3. FXIa Assay Mix: Prepare 50 µl of FXIa Assay Mix per well as given below:

40 μl FXIa Assay Buffer 10 μl FXIa Substrate

Mix well by pipetting up and down. Add 50 µl of FXIa Assay Mix to each well including Controls, FXIa Enzyme Positive Control, and Plasma Sample containing wells. *Do not add FXIa Assay Mix to pNA Standards*.

4. Measurement: For *p*NA Standards, measure the absorbance at 405 nm (OD405) in end point. For FXIa Enzyme, Sample Background Control and Plasma containing Samples, measure the absorbance at 405 nm (OD405) in kinetic mode for 0.5-1 h.

Notes:

- It is recommended to run at least 3-5 different amounts of Plasma samples to get accurate measurements of plasma FXIa activity.
- If plasma FXIa activity is low, higher amounts of chloroform-treated plasma can be activated with equal volume of FXIa activator and used in the assay.

5. Calculations:

- a. pNA Standard Curve: Obtain change in the absorbance ΔOD405 by subtracting absorbance of the 0 Standard Controls from those containing all standards. Plot the ΔO405 against nmol of pNA. The plot should be linear; determine the slope A (ΔOD405/nmol) of the curve.
- b. Plasma Samples: Use the linear region of kinetic progress curves to obtain slopes for all Activated Plasma containing reactions and Sample Background Control. Choose two time points (t₁ & t₂) in the linear range of the plot and obtain the corresponding values for the absorbance. Calculate ΔΟD405/Δt for each Activated Plasma Sample and corresponding Sample Background Control. Subtract ΔΟD405/Δt of the Sample Background Control from Activated Plasma Sample and obtain corresponding (B, ΔΟD405/min). Using this value, calculate Plasma FXIa activity in Plasma Equivalent Units per deciliter (PEU/dL) using following equation:

FXIa Activity
$$\left(\frac{PEU}{dL}\right) = \frac{B \times 1000 \times 100}{A \times C \times X}$$

where, $\mathbf{B} = \text{Plasma FXIa Activity as calculated } (\Delta \text{OD405/min}).$

 $X = \mu I$ of Plasma Sample used in the assay.

A = Slope of the pNA standard curve ($\Delta OD405/nmol$).

C = 140 (nmol/min/PEU); correction factor for the amount of pNA released under the assay conditions.

Unit Definition: 1 Loewy U/ml is the highest dilution of the enzyme capable of forming an insoluble clot under the conditions described by Loewy et al (*J. Bio. Chem.*, **1961**, <u>236</u>, 2625-2633); 1 PEU = 108 Loewy U.

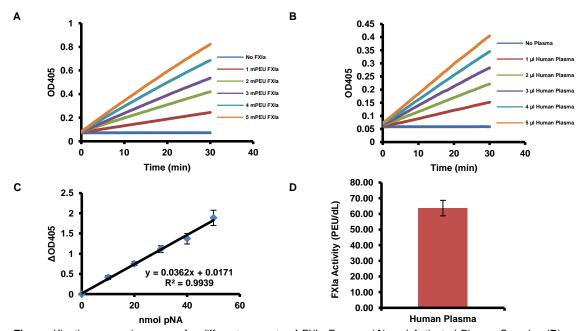


Figure: Kinetic progressive curves for different amounts of FXIa Enzyme (\mathbf{A}) and Activated Plasma Samples (\mathbf{B}) are shown. Standard curve for pNA (n = 3) (\mathbf{C}) was used to estimate FXIa activity in plasma (n = 5) (\mathbf{D}). Assays were performed according to the kit protocol.

VIII. RELATED PRODUCTS:

Factor Xa Activity Fluorometric Assay Kit (K361) Factor IXa Activity Assay Kit (Fluorometric) (K364) Factor XIIIa Activity Assay Kit (Colorimetric) (K522) Factor Xa Inhibitor Screening Kit (Fluorometric) (K362) Factor VIIIa Activity Assay Kit (Fluorometric) (K358)

FOR RESEARCH USE ONLY! Not to be used on humans.