



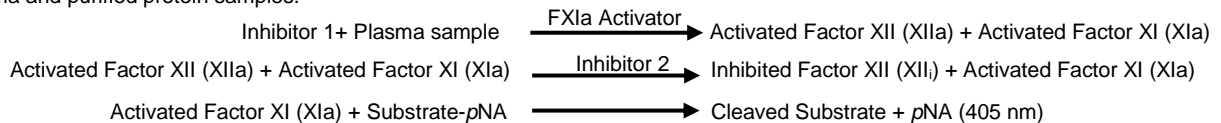
Factor XIa Activity Assay Kit (Colorimetric)

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 (*p*NA) 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
<i>p</i> NA Standard (0.1 M)	20 μ l	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 μ l 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 μ l of plasma in an Eppendorf tube and add 50 μ l 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 l of the chloroform treated plasma sample in an Eppendorf tube and add 1 μ l of Inhibitor 1. Incubate at room temperature (RT) for 10 min. Add 10 μ l 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 μ l 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 μ l of reconstituted FXIa enzyme solution. Bring the final volume in each well to 50 μ l with FXIa Assay Buffer.

2. ***p*NA Standard:** Dilute 5 μ l 0.1 M *p*NA Standard into 95 μ l FXIa Assay Buffer to prepare 5 mM *p*NA. Add 0, 2, 4, 6, 8, 10 μ l of 5 mM *p*NA standard into each well. Adjust volume to 100 μ l/well with FXIa Assay Buffer to generate 0, 10, 20, 30, 40, 50 nmol/well of *p*NA standard.

