Version 2 Last updated 6 September 2023

ab241037
Factor XIa Assay Kit

For the detection of enzymatic activities of factor XIa in plasma and purified protein samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Table of Contents

1. Overview 1
2. Protocol Summary 2
3. General guidelines, precautions, and troubleshooting 3
4. Materials Supplied, and Storage and Stability 4
5. Materials Required, Not Supplied 4
6. Reagent Preparation 5
7. Standard Preparation 6
8. Sample Preparation 7
9. Assay Procedure 8
10. Data Analysis 9
11. Typical Data 10
12. Notes 12

1. Overview

The Factor XIa Assay Kit (ab241037) 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.

2. Protocol Summary

Prepare all samples, controls and standards as instructed.

Prepare the standard curve using the 0.1 M pNA standard. Dilute to 5 mM by adding 5 µL into 95 µL of FXIa Assay Buffer.

Create the Assay Mix, add 50 µL to each well.

For the pNA standards measure the absorbance at 405 nm in end point mode.

For the FXIa Enzyme Sample Background Control and Plasma containing Samples, measure the absorbance at 405 nm in kinetic mode for 0.5-1 hour

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.
- Briefly centrifuge small vials prior to opening.

Item	Quantity	Storage condition
FXIa Assay Buffer	25 mL	4°C or -20°C
FXIa Activator	1 vial	-20°C
PK Inhibitor/Inhibitor 1	0.1 mL	-20°C
FXIIa Inhibitor/Inhibitor 2	0.1 mL	-20°C
FXIa Substrate	1 mL	-20°C
Human Factor XIa	1 vial	-20°C
pNA Standard I/pNA standard (0.1)	20 µL	-20°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

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

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 FXIa Assay Buffer:

Bring to room temperature before use.

6.2 FXIa Activator:

Bring to room temperature before use. After first use, this suspension can be stored at room temperature. Before each use mix well.

6.3 PK Inhibitor/Inhibitor 1:

Aliquot and store at -20°C. Avoid multiple freeze/thaw. Thaw on ice before use.

6.4 FXIIa Inhibitor/Inhibitor 2:

Aliquot and store at -20°C. Avoid multiple freeze/thaw. Thaw on ice before use

6.5 FXIa Substrate:

Aliquot and store at -20°C. Avoid multiple freeze/thaw. Thaw on ice before use

6.6 Human factor XIa:

Reconstitute with 20 µL of FXIa Assay Buffer. Store at -20°C. Avoid repeated freeze/thaw. Use within two months.

6.7 pNA Standard I/pNA Standard (0.1M):

Ready to use as supplied

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 Dilute 5 µL 0.1 M pNA Standard I/pNA Standard into 95 µL FXIa Assay Buffer to prepare 5 mM pNA.

7.2 Add 0, 2, 4, 6, 8 and 10 µL of the 5 mM pNA Standard I/pNA standard into each well.

7.3 Adjust volume to 100 µL/well with FXIa Assay Buffer to generate 0, 10, 20, 30, 40, 50 nmol/well of pNA Standard I/pNA standard.

Standard #	5 mM pNA Standard I/pNA standard (µL)	FXIa Assay Buffer (µL)	pNA concentration Per well (nmol)
1	10	90	50
2	8	92	40
3	6	94	30
4	4	46	20
5	2	98	10
6	0	100	0

8. Sample Preparation

ΔNote: The following pretreatment of plasma with chloroform is recommended but not mandatory.

8.1 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.
- Use 1-10 µL of the chloroform treated plasma sample in an Eppendorf tube and add 1 µL of PK Inhibitor/Inhibitor 1.
- Incubate at room temperature (RT) for 10 min. Add 10 µL 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.
- Add 1 µL of FXIIa Inhibitor/inhibitor 2 to the solution. Incubate further for 10 minutes at room temperature. Load this solution on a microplate well.
- 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

9. Assay Procedure

9.1 Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µL FXIa Assay Mix containing the following components. Mix well before use:

	FXIa Assay Mix
FXIa Assay Buffer	40 µL
Fxia Substrate	10 µL

9.2 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.

9.3 Do not add FXIa Assay Mix to pNA Standard I/pNA Standards.

9.4 Measurement:

- For pNA Standard I/pNA 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.

ΔNote: It is recommended to run at least 3-5 different amounts of Plasma samples to get accurate measurements of plasma FXIa activity.

ΔNote: 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.

10. Data Analysis

10.1 Subtract the 0 Standard reading from all Standard curve readings. Plot the background-subtracted pNA Standard I/pNA Standard Curve and calculate the slope.

10.2 If sample background control slope is significant, then subtract sample background control reading from sample readings.

10.3 Apply the corrected ΔOD405/Δt value to the pNA Standard I/pNA Standard Curve to get B nmol pNA in the sample well.

10.4 Using this value, calculate Plasma FXIa activity in Plasma Equivalent Units per deciliter (PEU/dL) using following equation:

$$\text{FXIa Activity } \frac{PEU}{dL} = \frac{B \times 1000 \times 100}{A \times C \times X}$$

Where:

B is the Plasma FXIa Activity as calculated (ΔOD405/min).

X is the µL of Plasma Sample used in the assay.

A is the Slope of the pNA Standard I/pNA standard curve (ΔOD405/nmol).

C is 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, 236, 2625-2633);

1 PEU = 108 Loewy U.

11. Typical Data

Typical data provided **for demonstration purposes only**.

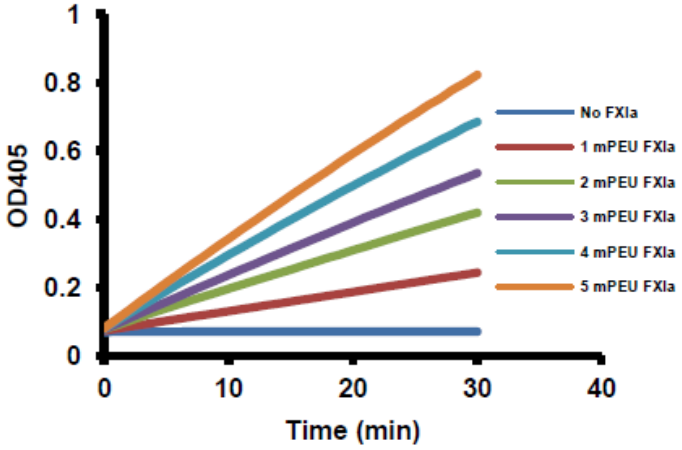

Figure 1. Kinetic progressive curves for varying amounts of FXIa Enzyme.

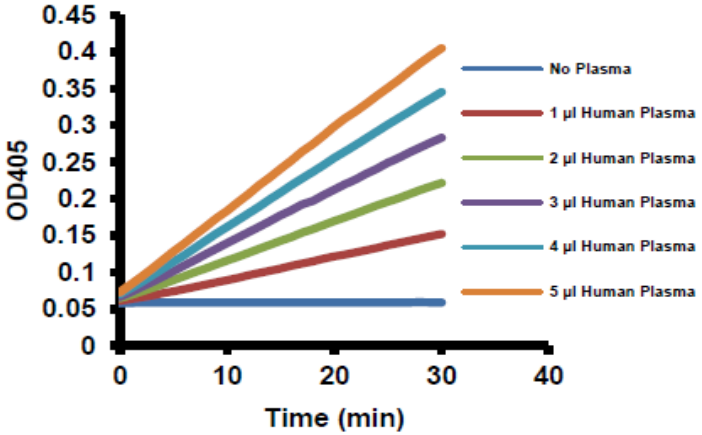

Figure 2. Kinetic progressive curves for different amounts of activated plasma samples.

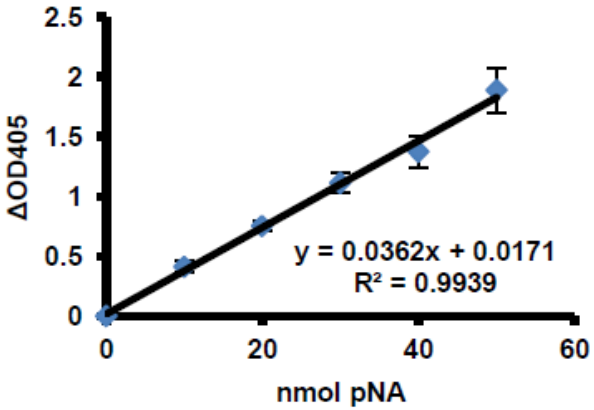

Figure 3: pNA Standard I/pNA Standard curve

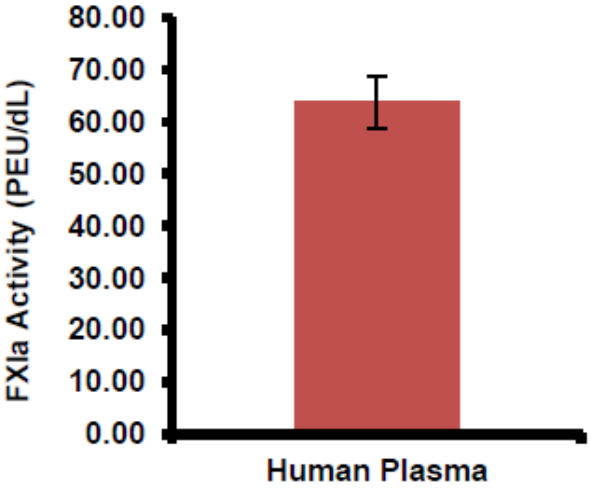

Figure 4: FXIa activity in human plasma.

12. Notes

Technical Support

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | 400 921 0189 / +86 21 2070 0500
Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abc.com | +64-(0)9-909-7829