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ab241041
Factor XII / XIIa Assay Kit

For the detection of enzymatic activities of Factor XII and Factor XIIa in plasma samples.

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

Table of Contents

1. Overview

The Factor XIIa Assay Kit (ab241041) utilizes the ability of factor XIIa to cleave a synthetic substrate to release p-Nitroaniline (pNA) which can be quantitatively measured by a colorimetric assay (OD405 nm). The kit is easy-to-use and can detect Factor XIIa (as low as 0.5 mU) from plasma samples. This kit also measures Factor XII. In the sample preparation, the FXII is converted to FXIIa by the Coagulation Activator Solution.

2. Protocol Summary

Prepare all samples, controls and standards as instructed.

Prepare the pNA standard curve.

Add 1-50 µL of sample to desired wells, adjust volume to 50 µL with Assay Buffer 35.

Prepare the Assay mix and add 50 µL to the sample wells.

For pNA Standards, measure the absorbance at 405 nm (OD405) in end point. For FXIIa Enzyme, Inhibitor Control and Plasma containing Samples, measure the absorbance at 405 nm (OD405) in kinetic mode for 0.5-1 h at 37 °C.

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.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

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.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition	Storage Condition (reconstituted)
Assay Buffer 35	25 mL	-20°C	4°C , -20°C
Coagulation Activator Solution	1 mL	-20°C	RT
Kallikrein Substrate	100 µL	-20°C	-20°C
Human Factor XIIa	1 vial	-20°C	-20°C
Human Prekallikrein	1 vial	-20°C	-20°C
FXIIa Inhibitor	100 µL	-20°C	-20°C
pNA Standard I	20 µL	-20°C	-20°C

PLEASE NOTE: Assay Buffer 35 was previously labeled as Assay Buffer XXXV and FXIIa Assay Buffer. The composition has not changed. Human Prekallikrein was previously labeled Factor XIIa Supplement, and is now supplied in a lyophilized state.

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 Assay Buffer 35:

Ready to use as supplied. Bring to room temperature (RT) before use.

6.2 Coagulation Activator Solution:

Bring to room temperature before use. The Coagulation Activator Solution may appear white due to it being a clay based material. If you see sediments, please mix well before use. After first use, it can be stored at room temperature.

6.3 Human Prekallikrein:

Reconstitute with 40 µL of ddH_2O. Aliquot and store at -20°C. Avoid multiple freeze/thaw. Thaw on ice before use.

6.4 FXIIa Inhibitor:

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

6.5 Human Factor XIIa:

Reconstitute with 100 µL of Assay Buffer 35 and store at -20°C. Avoid repeated freeze/thaw, use within two

months.

6.6 Kallikrein Substrate:

Thaw and bring to room temperature before use.

Store at -20°C.

6.7 pNA Standard I:

Thaw and bring to room temperature before use.

Store at -20°C.

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 of the 0.1 M pNA Standard I into 95 µL Assay Buffer 35 to prepare 5 mM pNA.

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

7.3 Adjust volume to 100 µL/well with Assay Buffer 35Buffer to generate 0, 10, 20, 30, 40, 50 nmol/well of pNA Standard I.

7.4 Mix well by pipetting.

Standard #	5 mM pNA Standard I (µL)	Assay Buffer 35 (µL)	pNA Standard I nmol/well
1	10	90	50
2	8	92	40
3	6	94	30
4	4	96	20
5	2	98	10
6	0	100	0

8. Sample Preparation

– For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

∆**Note:** The Following pretreatment of plasma with chloroform (8.1) is recommended but not mandatory.

Optional 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 16000 x g for 5 min to separate two layers.
– Carefully pipette top layer containing pretreated plasma in a separate Eppendorf tube.

∆**Note:** The pretreatment of plasma with chloroform is recommended but not mandatory.

8.1 Use 1-10 µL of the chloroform-treated or untreated plasma in an Eppendorf tube.

8.2 As an Inhibitor control, mix same volume of chloroform-treated or untreated plasma with 1 µL of FXIIa Inhibitor in a separate Eppendorf tube and incubate at RT for 10 min.

8.3 To each Eppendorf tube, add 10 µL of Coagulation Activator Solution and mix well by gentle tapping on the tube.

8.4 Incubate at 37°C for 5 min (or on ice for 45 min). Transfer the entire solution to a microplate well.

8.5 Bring the final volume in each well to 50 µL with Assay Buffer 35.

Optional: Centrifuge the tube at 3000 x g for 5 min and separate the solution from the Coagulation Activator Solution. Load the supernatant solution on a microplate well. Bring the final volume in each well to 50 µL with Assay Buffer 35. While this step improves light scattering, Coagulation Activator Solution could bind some FXIIa.

Positive Control:

8.6 As a Positive Control, use 1-5 µL of reconstituted FXIIa enzyme solution in separates well with and without 1 µL of FXIIa Inhibitor.

8.7 Incubate at RT for 10 min.

8.8 Add 2.0 µl of Human Prekallikrein solution

ΔNote: Human Prekallikrein is not required for plasma containing samples.

8.9 Bring the final volume in each well to 50 µL with Assay Buffer 35.

9. Assay Procedure

9.1 **FXIIa Assay Mix:** Prepare 50 µL of FXIIa Assay Mix per well as given below:

	Assay Mix
Assay Buffer 35	49 µL
Kallikrein Substrate	1 µL

9.2 Mix well by pipetting up and down. Add 50 µL of FXIIa Assay Mix to each well including Inhibitor Control, FXIIa Enzyme Positive Control, and Plasma Sample containing wells. Do not add FXIIa Assay Mix to pNA Standards.

9.3 **Measurement:** For pNA Standards, measure the absorbance at 405 nm (OD405) in end point. For FXIIa Enzyme, Inhibitor Control and Plasma containing Samples, measure the absorbance at 405 nm (OD405) in kinetic mode for 0.5-1 h at 37 °C.

Δ**Note:** It is recommended to run at least 3-5 different amounts of Plasma samples to get accurate measurements of plasma FXIIa activity.
Δ**Note:** If plasma FXIIa activity is low, higher amounts of chloroform-treated or untreated plasma can be activated with equal volume of Coagulation Activator Solution and used in the assay.

10. Data Analysis

These alternate calculations are offered to define activity in terms of pNA released from the substrate.

10.1 Subtract the 0 Standard reading from all Standard curve readings. Plot the background-subtracted pNA Standard and calculate the equation of the line.

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

10.3 Find two time points (T_1, T_2) in the linear range for Factor XIIa and calculate $\Delta OD = A_2 - A_1$.

10.4 Determine the amount of pNA released by applying the ΔOD to the equation of the line and solving for X.

10.5 Using this value, calculate Plasma FXIIa activity mU/ml using following equation:

$$\textbf{FXIIa Activity} = \left(\frac{X}{\Delta T * V}\right) * D = \textbf{mU/mL}$$

Where:

X is the nmol of pNA released

V is ml of Plasma Sample used in the assay

ΔT is the change in time ($\Delta T = T_2 - T_1$) for the points in the linear range

D is the dilution factor if samples were diluted before adding to the well.

Unit Definition: 1 Unit releases 1 µmol of pNA/min under the assay conditions.

11. Alternate Data Analysis (Legacy)

11.1 Obtain the absorbance ΔOD405 by subtracting absorbance of the 0 Standard from all standards. Plot the ΔO405 against nmol of pNA. The plot should be linear; determine the slope A (ΔOD405/nmol) of the curve.

11.2 Use the linear region of kinetic progress curves to obtain slopes for all Activated Plasma containing reactions and Inhibitor Control.

11.3 Choose two time points (t1 & t2) in the linear range of the plot and obtain the corresponding values for the absorbance.

11.4 Calculate ΔOD405/Δt for each Activated Plasma Sample and corresponding Inhibitor Control. Subtract ΔOD405/Δt of the Inhibitor Control from Activated Plasma Sample and obtain corresponding (B, ΔOD405/min). Using this value, calculate Plasma FXIIa activity in Plasma Equivalent Units per deciliter (PEU/dL) using following equation:

$$\textbf{FXIIa Activity PEU/dL} = \frac{\mathbf{B \times 1000 \times 100}}{\mathbf{A \times C \times X}}$$

Where:

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

X is µl of Plasma Sample used in the assay

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

C is 190 (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.

12. Typical Data

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

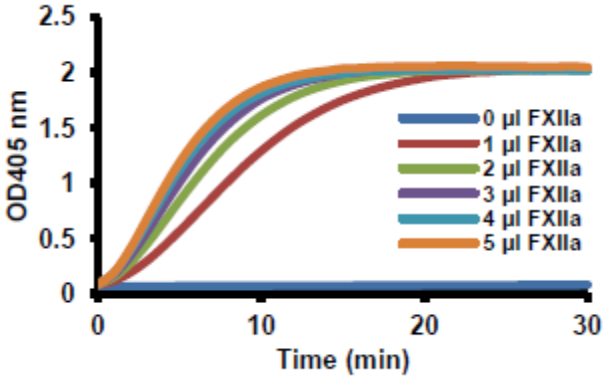

Figure 1. Kinetic progressive curves for different amounts of FXIIa Enzyme.

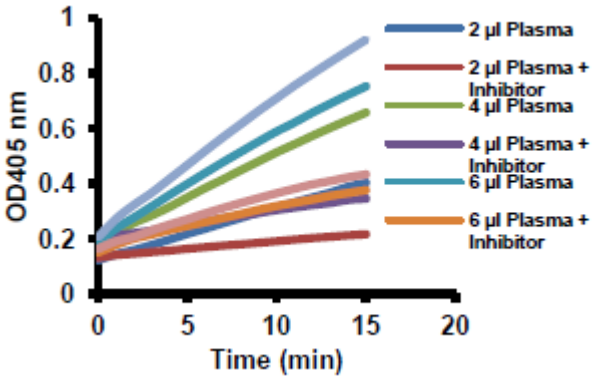

Figure 2. Kinetic progressive curves for different amounts of are shown. Standard curve for pNA (n = 3).

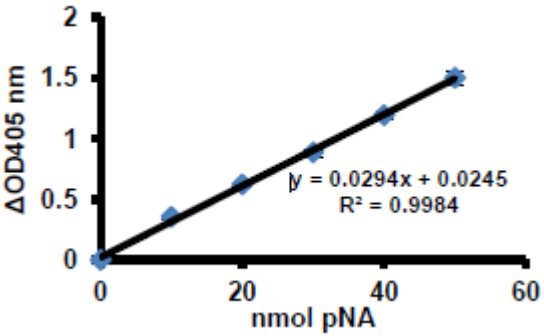

Figure 3. Standard curve for pNA (n = 3).

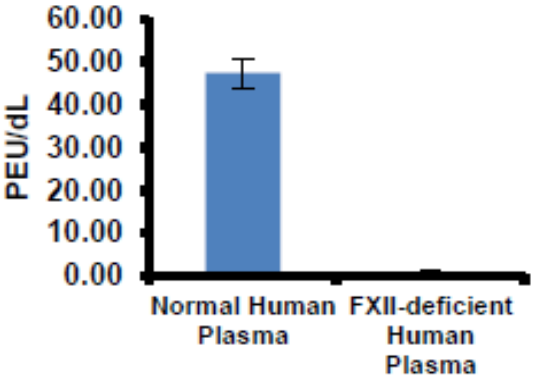

Figure 4. was used to estimate FXIIa activity in Normal Pooled Human Plasma and FXII-deficient Human Plasma (n = 3).

13. Notes

Technical Support

For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)