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Factor XIIa Activity Assay Kit (Colorimetric)

09/16

(Catalog # K994-100; 100 assays, Store kit at -20°C)

I. Introduction:

Factor XII or Hageman factor (EC 3.4.21.38), is the zymogen form of factor XIIa, a serine protease involved in Coagulation Pathway. This single chain zymogen is activated by Kallikrein into a two-chain serine protease (XIIa) with a heavy chain (factor XIIa-alpha) and a light chain. Human Factor XII and Prekallikrein are thought to be involved in a reciprocal activation mechanism in which Factor XIIa activates Prekallikrein to Kallikrein, which in turn converts Factor XII to XIIa. Factor XIIa activates Factor XI to XIa thereby triggering the Contact Factor cascade. The defects in this gene do not cause any clinical symptoms but prolong the whole blood clotting time. BioVision's Factor XIIa activity assay kit utilizes the ability of factor XIIa to cleave a synthetic substrate to release *p*-Nitroaniline (*p*NA) 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 1 mPEU) from plasma samples.

Plasma sample (Factor XII) —FXIIa Activator→ Activated Factor XII (XIIa)

Activated Factor XII (XIIa) + Prekallikrein (from Plasma) → Kallikrein

Kallikrein + Substrate-*p*NA → Cleaved Substrate + *p*NA (405 nm)

II. Applications:

- Detection of enzymatic activities of factor XIIa in plasma samples

III. Sample Type:

- Plasma samples

IV. Kit Contents:

Components	K994-100	Cap Code	Part Number
FXIIa Assay Buffer	25 ml	WM	K994-100-1
FXIIa Activator	1 ml	Clear	K994-100-2
FXIIa Substrate	0.1 ml	Red	K994-100-3
Human Factor XIIa	1 Vial	Green	K994-100-4
Human Factor XIIa Supplement	10 µl	Blue	K994-100-5
FXIIa Inhibitor	0.1 ml	Orange	K994-100-6
*p*NA Standard (0.1 M)	20 µl	Yellow	K994-100-7

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.

- **FXIIa Assay Buffer**: Bring to room temperature before use. Store at 4°C or -20°C.
- **FXIIa Activator**: Bring to room temperature before use. After first use, it can be stored at room temperature. Before each use, mix well.
- **Human Factor XIIa Supplement and FXIIa Inhibitor**: Aliquot and store at -20°C. Avoid multiple freeze/thaw. Thaw on ice before use.
- **Human Factor XIIa**: Reconstitute with 100 µl of FXIIa Assay Buffer and store at -20°C. Avoid repeated freeze/thaw, use within two months.
- **FXIIa Substrate and *p*NA Standard:** Ready to use. Store at -20°C.

VII. FXIIa Activity Assay Protocol:

1. Sample Preparation: *The following pretreatment of plasma with chloroform (1.a) 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 16000 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 µl of the chloroform-treated or untreated plasma in an Eppendorf tube. As an Inhibitor control, mix same volume of plasma with 1 µl of FXIIa Inhibitor in a separate Eppendorf tube and incubate at RT for 10 min.

c) To each Eppendorf tube, add 10 µl of FXIIa Activator and mix well by gentle tapping on the tube. Incubate at 37°C for 5 min (or on ice for 45 min). Transfer the entire solution to a microplate well. Bring the final volume in each well to 50 µl with FXIIa Assay Buffer.

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

d) As a Positive Control, use 1-10 µl of reconstituted FXIIa enzyme solution in separates well with and without 1 µl of FXIIa Inhibitor. Incubate at RT for 10 min. Add 1.0 µl of Human Factor XIIa Supplement solution (**Do not add Human Factor XIIa Supplement to plasma containing samples**). Bring the final volume in each well to 50 µl with FXIIa Assay Buffer.

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

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

49 µl FXIIa Assay Buffer
1 µl FXIIa Substrate

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*.

4. **Measurement**: For *p*NA 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.

Notes:

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

5. **Calculations:**

a. ***p*NA Standard Curve**: Obtain the absorbance ΔOD405 by subtracting absorbance of the 0 Standard from all standards. Plot the ΔO405 against nmol of *p*NA. 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 Inhibitor Control. Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values for the absorbance. 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 **P**lasma **E**quivalent **U**nits per deciliter (**PEU/dL**) using following equation:

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

where, **B** = Plasma FXIIa Activity as calculated (ΔOD405/min).
X = µl of Plasma Sample used in the assay.
A = Slope of the *p*NA standard curve (ΔOD405/nmol).
C = **190** (nmol/min/PEU); correction factor for the amount of *p*NA 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.

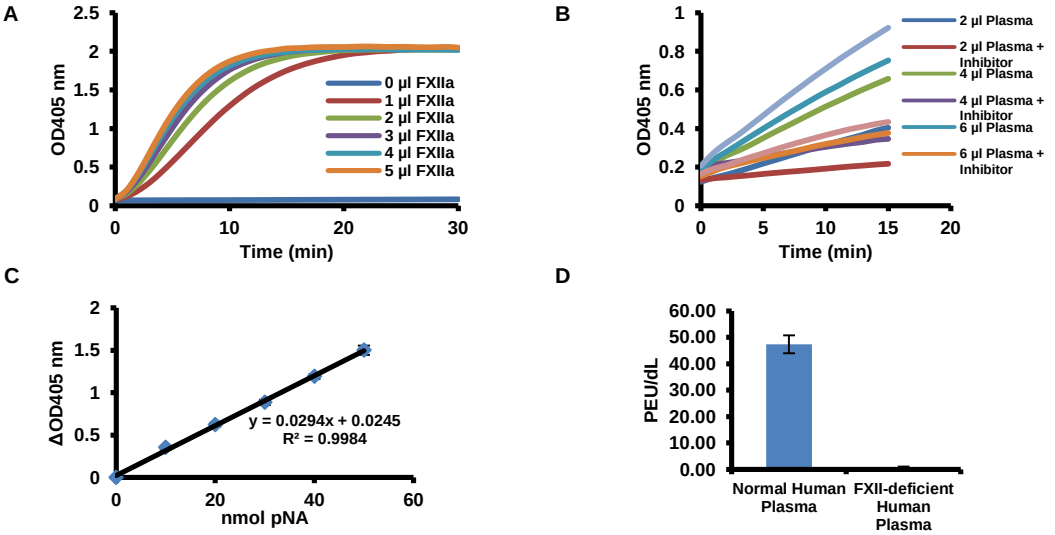

Figure: Kinetic progressive curves for different amounts of FXIIa Enzyme (**A**) and Activated Plasma Samples (**B**) are shown. Standard curve for *p*NA (n = 3) (**C**) was used to estimate FXIIa activity in Normal Pooled Human Plasma and FXII-deficient Human Plasma (n = 3) (**D**). Assays were performed according to the kit protocol.

VIII. RELATED PRODUCTS:

Factor Xa Activity Fluorometric Assay Kit (K361)
Factor Xa Inhibitor Screening Kit (Fluorometric) (K362)
Factor IXa Activity Assay Kit (Fluorometric) (K364)
Factor VIIIa Activity Assay Kit (Fluorometric) (K358)
Factor XIIIa Activity Assay Kit (Colorimetric) (K522)
Factor XIa Activity Assay Kit (Colorimetric) (K973)

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