Factor XIIIa Activity Assay Kit (Colorimetric) (#BN00754)

(Catalog # BN00754; 100 assays, Store kit at -20°C)

I. Introduction:

Factor XIII or fibrin stabilizing factor (EC 2.3.2.13) is an enzyme involved in the blood coagulation system that crosslinks fibrin. Factor XIII is a transglutaminase that circulates in the plasma as a heterotetramer of two catalytic "A" subunits and two carrier "B" subunits. Factor XIII is activated by thrombin in the presence of calcium which leads to the release of the activation peptide, followed by the dissociation of carrier subunits. The activated Factor XIII (XIIIa) acts on fibrin (Factor Ia) to form γ-glutamyl-Ɛ-lysyl amide cross links between fibrin molecules leading to the formation of an insoluble clot. The deficiency of Factor XIII affects fibrin clot stability and bleeding disorders. Assay Genie's Factor XIIIa activity assay kit utilizes the transglutaminase activity of factor XIIIa to cross link an amine-containing substrate to glutamine-containing substrate resulting in the loss of ammonia which can be quantitatively measured by a colorimetric assay. The kit is easy-to-use and can detect Factor XIIIa (as low as 0.1 Loewy U) from plasma and purified protein samples.

Factor XIII (or Plasma sample) ⟶ Activated Factor XIII (XIIIa)

Glutamine Substrate + Amine Substrate —XIIIa⟶ Cross-linked substrates + NH_4^+

NH_4^+ + Detection Reagent ⟶ Decrease in Absorbance (340 nm)

II. Applications:

- Detection of enzymatic activities of factor XIII in plasma and purified protein samples

III. Sample Type:

- Plasma and purified protein samples

IV. Kit Contents:

Components	BN00754	Cap Code	Part Number
FXIIIa Assay Buffer	2.5 ml	NM	BN00754-1
FXIIIa Activation Buffer (4X)	2.5 ml	NM-Brown	BN00754-2
FXIIIa Reaction Buffer (4X)	2.5 ml	Brown	BN00754-3
FXIIIa Detection Buffer (2X)	5 ml	NM/Blue	BN00754-4
FXIIIa Probe	0.2 ml	Red	BN00754-5
Human Factor XIIIa (0.5 Loewy U/µl)	20 µl	Green	BN00754-6
NH_4Cl Standard (10 mM)	100 µl	Yellow	BN00754-7
FXIIIa Inhibitor (Iodoacetamide)	1 Vial	Amber	BN00754-8

V. User Supplied Reagents and Equipment:

- 96-well clear well plate (preferably UV-transparent, e.g. Corning® 96 Well Clear Flat Bottom UV-Transparent Microplate)
- Multi-well spectrophotometer

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. High concentrations of ammonia in the samples or buffers will interfere with the performance of the kit. Avoid exposing the entire kit or reagents to ammonia or ammonia producing reagents.

- **FXIIIa Assay Buffer**: Bring to room temperature before use. Store at 4°C or -20°C.
- **FXIIIa Activation, Reaction and Detection Buffers**: Thaw on ice before use. Store at -20°C. If needed, aliquot into smaller volumes for storage at -20°C. Avoid repeated freeze/thaw. Use within two months.
- **FXIIIa Probe**: Aliquot and store at -20°C. Thaw on ice before use. Avoid repeated freeze/thaw. Use within two months.
- **Human Factor XIIIa**: Store at -20°C. Thaw on ice before use. Avoid repeated freeze/thaw. Use within two months.
- **FXIIIa Inhibitor**: Reconstitute in 1 ml of ddH_2O to obtain 100 mM Iodoacetamide. Aliquot and store at -20°C. Thaw on ice before use.

VII. FXIIIa Activity Assay Protocol:

1. **Sample Preparation:** Use 5-25 µl of clarified plasma in a well and bring the final volume to 25 µl with FXIIIa Assay Buffer. If necessary, dilute with FXIIIa Assay Buffer. For positive control, dilute 10 µl of FXIIIa enzyme solution with 40 µl of FXIIIa Assay buffer to obtain 0.1 Loewy U/µl of the enzyme solution. Use 10 µl of diluted 0.1 Loewy U/µl enzyme solution in a well of a 96-well clear microplate. Bring the final volume in each well to 25 µl with FXIIIa Assay Buffer. Mix well.
2. **NH_4Cl Standard**: Use 0, 1, 2, 3, 4 and 5 µl of the provided 10 mM ammonium chloride (NH_4Cl) solution in a well to obtain 0 (**Control**), 10, 20, 30, 40 and 50 nmol of NH_4Cl per well. Bring the final volume to 25 µl with FXIIIa Assay Buffer.
3. **FXIIIa Assay Mix**: Prepare 100 µl of FXIIIa Assay Mix per well as given below:

    25 µl FXIIIa Activation Buffer
    25 µl FXIIIa Reaction Buffer
    48 µl FXIIIa Detection Buffer
    2 µl FXIIIa Probe

Mix well by pipetting up and down. Add 100 µl of FXIIIa Assay Mix to each well including Controls, NH_4Cl Standards and FXIIIa Enzyme Positive Control, and Plasma Sample containing wells. Mix well by pipetting up and down without creating any bubbles.

4. **Measurement**: For NH_4Cl Standards, measure the absorbance at 340 nm (OD340) in kinetic mode for 30 min or end point after 30 min. For FXIIIa Enzyme Standards and Plasma Samples, measure the absorbance at 340 nm (OD340) in kinetic mode for 1-2 h.

   Notes:

   - FXIIIa standard curve for higher concentrations (0-2 Loewy U) can be generated by using appropriate amount of diluted FXIIIa Enzyme Standard in the assay.
   - As a control for plasma samples, a parallel Iodoacetamide sample background control containing same amount of plasma and FXIIIa Assay Mix with 1 µl of 100 mM Iodoacetamide solution must be used.

5. **Calculations:**

   a. **NH_4Cl Standard Curve**: Obtain change in the absorbance ΔOD340 (after 30 min) by subtracting absorbance of the 0 Standard Control from those containing all standards. Plot the absolute value of ΔOD340 (│ΔOD340│) against nmol of NH_4Cl. The plot should be linear; determine the slope **A** (│ΔOD340│/nmol) of the curve.

   b. **Plasma Samples**: Use the linear region of kinetic progress curves to obtain slopes for all Plasma Sample containing reactions and Iodoacetamide control. Choose two time points (t1 & t2) in the linear range of the plot and obtain the corresponding values for the absorbance. Calculate │ΔOD340│/Δt for each Plasma Sample and corresponding Iodoacetamide Control. Subtract │ΔOD340│/ΔT of the Iodoacetamide control from Plasma Sample and obtain corresponding (**B**, **│ΔOD340│/min**). Using this value, calculate sample FXIIIa activity in **P**lasma **E**quivalent **U**nits per deciliter (**PEU/dL**) from following equation:

$$\textbf{FXIIIa Activity}\left(\frac{PEU}{dL}\right)=\frac{B\times 1000\times 100}{A\times C\times X\times 108}$$

where, **B** = Plasma FXIIIa Activity as calculated (│ΔOD340│/min).
X = µl of Plasma Sample used in the assay.
A = Slope of the NH_4Cl standard curve (│ΔOD340│/nmol).
C = **1.2** (nmol/Loewy U, Correction factor for the amount of ammonia 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.

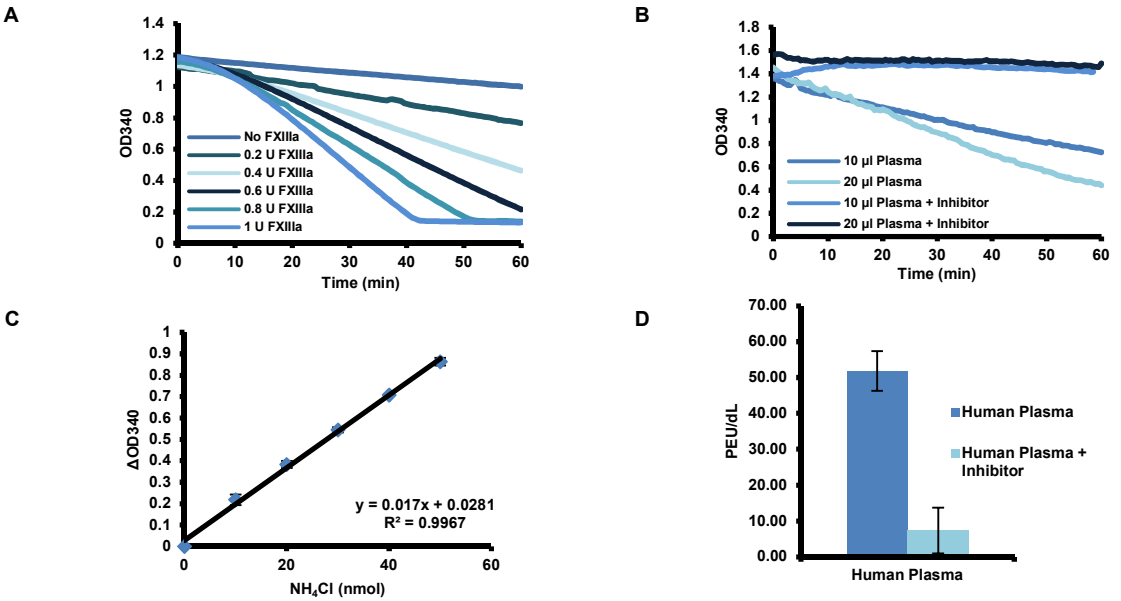

Figure: Kinetic progressive curves for different amounts of FXIIIa Enzyme (**A**), and Plasma Samples with and without Iodoacetamide control (**B**) are shown. Standard curves for NH_4Cl (n = 3) (**C**) was used to estimate FXIIIa activity in plasma samples (n = 3) (**D**). Assays were performed according to the kit protocol.

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