



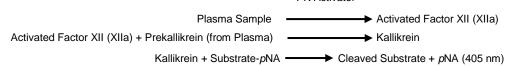
# Plasma Kallikrein Activity Assay Kit (Colorimetric)

09/16

(Catalog # K997-100; 100 assays, Store kit at -20°C)

#### I. Introduction:

Plasma Prekallikrein (EC 3.4.21.34), is the glycosylated single chain zymogen precursor of the plasma serine protease Kallikrein. It circulates with kininogen and is activated by Factor XIIa to Kallikrein in the intrinsic coagulation pathway. Kallikrein activates plasminogen in fibrinolysis and cleaves kininogen in the bradykinin system of vasodilation. Prekallikrein deficiency is rare and causes increased activated partial thromboplastin time. Elevated plasma Prekallikrein is associated with diabetes and cardiovascular disease. Plasma Kallikrein inhibitors have been proposed as drugs to manage Hereditary Angioedema. BioVision's Plasma Kallikrein Activity Assay Kit utilizes the ability of active Plasma Kallikrein to cleave a synthetic pNA-based peptide substrate to release pNA (OD405 nm), which can be easily quantified using a microplate reader. The Plasma Kallikrein Specific Inhibitor (PKSI) selectively inhibits the ability of Plasma Kallikrein to the cleave the synthetic substrate. The kit is easy-to-use and can detect PK activity of Purified Plasma Kallikrein and Plasma Samples.



## II. Applications:

Detection of enzymatic activities of Plasma Kallikrein in plasma samples

#### III. Sample Type:

- Plasma samples
- Purified Kallikrein

#### IV. Kit Contents:

Components	K997-100	Cap Code	Part Number
PK Assay Buffer	25 ml	WM	K997-100-1
PK Activator	1 ml	Clear	K997-100-2
PK Substrate	0.1 ml	Red	K997-100-3
Human PK	1 Vial	Green	K997-100-4
PKSI Inhibitor	0.1 ml	Orange	K997-100-5
pNA Standard (0.1 M)	20 µl	Yellow	K997-100-6

#### 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.

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

# VII. PK Activity Assay Protocol:

- 1. Sample Preparation: The following pretreatment of plasma with chloroform 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 plasma sample in an Eppendorf tube. As an Inhibitor control, preincubate same volume of plasma with 1 µl of PKSI Inhibitor in a separate Eppendorf tube at RT for 10 min.
  - c) To each Eppendorf tube, add 10 µl of PK Activator solution and mix well by gentle tapping the tube. Incubate at 37°C for additional 5 min (or on ice for 45 min). Transfer this entire solution to a microplate well. Bring the final volume in each well to 50 µl with PK Assay Buffer.

**Optional**: Centrifuge the tube at 3000 x g for 5 min and remove the solution from activator. Load this solution on a microplate well. Bring the final volume in each well to 50  $\mu$ l with PK Assay Buffer.

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- d) As a Positive Control, use 1-20 μl of reconstituted human PK enzyme solution in a separate well with and without 1 μl of PKSI Inhibitor. Incubate at RT for 10 min. Bring the final volume in each well to 50 μl with PK Assay Buffer.
- 2. pNA Standard: Dilute 5 μl 0.1 M pNA Standard into 95 μl PK Assay Buffer to prepare 5 mM pNA. Add 0, 2, 4, 6, 8, 10 μl of 5 mM pNA standard into each well. Adjust volume to 100 μl/well with PK Assay Buffer to generate 0, 10, 20, 30, 40, 50 nmol/well of pNA standard.
- 3. PK Assay Mix: Prepare 50 µl of PK Assay Mix per well as given below:

49 µl PK Assay Buffer 1 µl PK Substrate

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

**4. Measurement**: For pNA Standards, measure the absorbance at 405 nm (OD405) in end point. For PK 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 PK activity.
- If plasma PK activity is low, higher amounts of chloroform-treated plasma can be activated with equal volume of PK activator and
  used in the assay.

# 5. Calculations:

- a. pNA Standard Curve: Obtain change in the absorbance ΔOD405 by subtracting absorbance of the 0 Standard Controls from those containing all standards. Plot the ΔO405 against nmol of pNA. 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<sub>1</sub> & t<sub>2</sub>) in the linear range of the plot and obtain the corresponding values for the absorbance. Calculate ΔΟD405/Δt for each Activated Plasma Sample and corresponding Inhibitor Control. Subtract ΔΟD405/Δt of the Inhibitor Control from Activated Plasma Sample and obtain corresponding (B, ΔΟD405/min). Using this value, calculate Plasma PK activity using following equation:

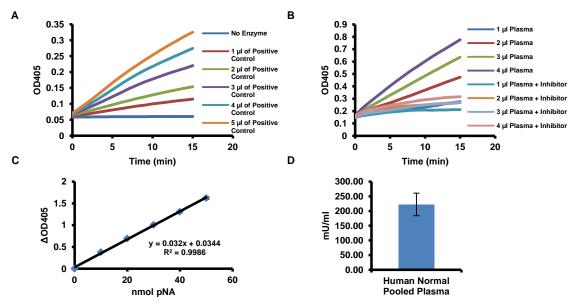
PK Activity 
$$\left(\frac{mU}{ml}or\frac{U}{L}\right) = \frac{B \times 1000}{A \times X}$$

where,  $\mathbf{B} = \text{Plasma PK Activity as calculated } (\Delta \text{OD405/min}).$ 

 $X = \mu I$  of Plasma Sample used in the assay.

A = Slope of the pNA standard curve ( $\Delta OD405/nmol$ ).

Unit Definition: 1 U is the amount of Plasma Kallikrein required to hydrolyze one µmole of PK Substrate per minute under the assay conditions.



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

#### **VIII. RELATED PRODUCTS:**

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