



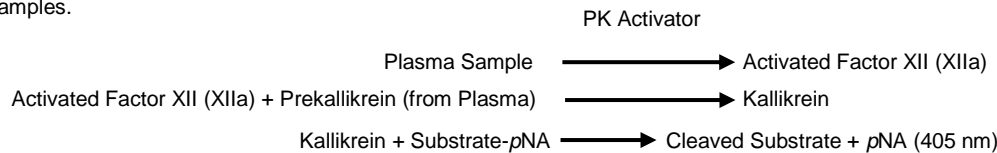
Plasma Kallikrein Activity Assay Kit (Colorimetric)

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 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 μ l	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 μ l 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.*

- Chloroform Pretreatment:** Take 50 μ l of plasma in an Eppendorf tube and add 50 μ l 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.
- Use 1-10 μ l of the chloroform treated plasma sample in an Eppendorf tube. As an Inhibitor control, preincubate same volume of plasma with 1 μ l of PKSI Inhibitor in a separate Eppendorf tube at RT for 10 min.
- To each Eppendorf tube, add 10 μ l 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 μ l 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 μ l with PK Assay Buffer.

