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Plasma Kallikrein Inhibitor Screening Kit (Colorimetric)

10/18

(Catalog # K989-100; 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 (PK). 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 Inhibitor Screening Kit utilizes the ability of active Plasma Kallikrein to cleave a synthetic *p*NA-based peptide substrate to release *p*NA (OD405 nm), which can be easily quantified using a microplate reader. In the presence of a Plasma Kallikrein inhibitor, the cleavage of this substrate is reduced/abolished resulting in decrease or total loss of the pNA absorbance. This simple and high-throughput adaptable assay kit can be used to screen/study/characterize potential inhibitors of Plasma Kallikrein.

PK Substrate-*p*NA $\xrightarrow{\text{PK}}$ Cleaved Substrate + *p*NA (OD405 nm)

PK Substrate-*p*NA $\xrightarrow[\text{+ PK Inhibitor}]{\text{PK}}$ Decrease/Loss in *p*NA Absorbance (OD405 nm)

II. Applications:

- Screening/characterizing inhibitors of Plasma Kallikrein.

III. Kit Contents:

Components	K989-100	Cap Code	Part Number
PK Assay Buffer	25 ml	WM	K989-100-1
Human PK	1 Vial	Green	K989-100-2
PK Substrate	1 ml	Red	K989-100-3
PK Inhibitor (1 mU/µl)	0.1 ml	Blue	K989-100-4

IV. User Supplied Reagents and Equipment:

- 96-well clear well plate
- Multi-well spectrophotometer

V. 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 Inhibitor**: Aliquot and store at -20°C. Avoid multiple freeze/thaw. Thaw on ice before use.
- **Human PK**: Reconstitute with 1.1 ml of PK Assay Buffer and store at -20°C. Avoid repeated freeze/thaw, use within two months.

VI. PK Inhibitor Screening Protocol:

1. **PK Enzyme Working Solution Preparation**: For each well (Enzyme Control-EC, Sample-S, Inhibitor Control-IC), prepare 40 µl of PK Enzyme Working Solution.

EC, S and IC	Background Control (BC)
30 µl PK Assay Buffer	40 µl PK Assay Buffer
10 µl Reconstituted PK enzyme solution	----

2. **Compounds, Inhibitor Control & Enzyme Control Preparations:** Dissolve candidate inhibitors at 100X highest final test concentration using preferred solvent. Dilute to 10X the desired test concentration with PK Assay Buffer. Add 10 µl test inhibitors (**S**) or PK Assay Buffer (**EC** or **BC**). For Inhibitor Control (**IC**), add 10 µl PK Inhibitor into PK enzyme containing well(s). Incubate at RT for 15 min.

   Note: Many commonly-used organic solvents can severely impact enzymatic activity. Importantly, DMSO may cause significant inhibition of kallikrein at final concentrations of ≥10% (v/v). We recommend preparing a parallel solvent control (**SC**) well with the same final concentration of solvent used to solubilize test ligands where the 30µl of assay buffer in EC are substituted with solvent control.

3. **PK Substrate Mix**: Prepare 50 µl of PK Substrate Mix per well as given below:

   40 µl PK Assay Buffer
   10 µl PK Substrate

   Dissolve the Substrate Mix by vigorous vortexing for 5 min. Centrifuge at 14000 x g for 5 min to remove any undissolved material. Add 50 µl of PK Substrate Mix to Background Control (BC), Enzyme Control (EC), Inhibitor Control (IC), SC, & Sample (S) wells. Mix well.

4. **Measurement**: Measure the absorbance at 405 nm (OD405) in kinetic mode for 0.5-1 h at 37 °C.

5. **Calculations:** Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values for the OD405 (OD_1 and OD_2). Calculate the slope (ΔOD405/Δt) for all samples and Enzyme Control (use the value of SC well(s) instead of EC if it is significantly different from EC value).

$$\% \textbf{ Inhibition} = \frac{\textbf{Slope of EC} - \textbf{Slope of S}}{\textbf{Slope of EC}} \times 100$$

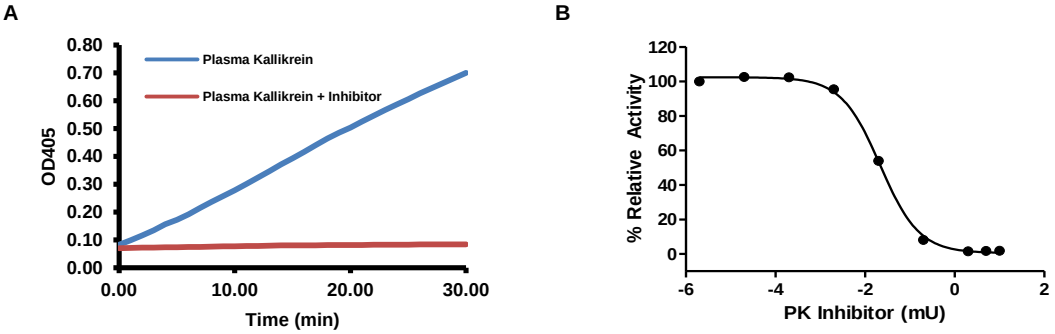

Figure: (a) Progress curve of Plasma Kallikrein activity in the presence or absence of the PK Inhibitor. (b) IC_{50} of PK Inhibitor against Plasma Kallikrein activity was 21.7 ± 3 µU (n = 3). Assays were performed according to the kit protocol.

VII. 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)

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