Version 4a, Last updated 19 May 2025

# ab241042 Plasma Kallikrein Assay Kit

For the measurement of Plasma Kallikrein in plasma samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

# Table of Contents

# 1. Overview

Plasma Kallikrein Assay Kit (ab241042) utilizes the ability of active Plasma Kallikrein to cleave a synthetic *p*NA-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.

Prepare Samples and pretreat with chloroform (recommended but not mandatory) as directed.

Prepare Standards as directed.

Prepare Reaction Mix. Add to samples, and controls, as appropriate.

Measure absorbance at OD 405 nm in kinetic mode for 0.5-1 h at 37°C.

## 2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

| Item | Quantity | Storage temperature (before prep) | Storage temperature (after prep) |
|---|---|---|---|
| Assay Buffer XXXV/PK Assay Buffer | 25 mL | -20°C | 4°C or -20°C |
| Coagulation Activator Solution/PK Activator | 1 mL | -20°C | RT |
| Kallikrein Substrate/PK Substrate | 0.1 mL | -20°C | -20°C |
| Native Human PK/Human PK | 1 vial | -20°C | -20°C |
| PKSI Inhibitor | 0.1 mL | -20°C | -20°C |
| pNA Standard I/*p*NA Standard (0.1 M) | 20 µL | -20°C | -20°C |

Native Human PK was previously called Recombinant Human PK. Formulation has not changed

## 3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96-well clear well plate.
- Multi-well spectrophotometer.
- Chloroform.

## 4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

# 5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

### 5.1 Assay Buffer XXXV/PK Assay Buffer

Ready to use as supplied. Warm to room temperature before use. Store at 4°C or -20°C.

### 5.2 Coagulation Activator Solution/Coagulation PK Activator

Ready to use as supplied. Bring to room temperature before use. After first use, it can be stored at room temperature. Before each use, mix well.

### 5.3 Kallikrein Substrate/PK Substrate

Ready to use as supplied. Store at -20°C.

### 5.4 PKSI Inhibitor

Aliquot and store at -20°C. Avoid multiple freeze/thaw. Thaw on ice before use.

### 5.5 Native Human PK/Human PK

Reconstitute with 100 µL of Assay Buffer XXXV/PK Assay Buffer and store at -20°C. Avoid repeated freeze/thaw, use within two months.

### 5.6 pNA Standard I/*p*NA Standard

Ready to use as supplied. Store at -20°C.

## 6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Dilute 5 µL 0.1 M pNA Standard I/*p*NA Standard into 95 µL Assay Buffer XXXV/PK Assay Buffer to prepare 5 mM *p*NA.
2. Add 0, 2, 4, 6, 8, 10 µL of 5 mM pNA Standard/*p*NA standard into each well.
3. Adjust volume to 100 µL/well with Assay Buffer XXXV/PK Assay Buffer to generate 0, 10, 20, 30, 40, 50 nmol/well of pNA Standard I/*p*NA standard.

| Standard # | 5 mM pNA Standard I/*p*NA Standard (µL) | Assay Buffer XXXV/PK Assay Buffer (µL) | Final volume standard in well (µL) | *p*NA (nmol/well) |
|---|---|---|---|---|
| 1 | 0 | 100 | 100 | 0 |
| 2 | 2 | 98 | 100 | 10 |
| 3 | 4 | 96 | 100 | 20 |
| 4 | 6 | 94 | 100 | 30 |
| 5 | 8 | 92 | 100 | 40 |
| 6 | 10 | 90 | 100 | 50 |

# 7. Sample Preparation

## General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay.

**ΔNote:** The following pretreatment of plasma with chloroform is recommended but not mandatory.
The chloroform treatment of plasma rapidly inactivates endogenous plasma kallikreinogen inhibitors without affecting the concentration of plasma kallikreinogen. Chloroform destroys the inhibitors of the kinin-forming factors and thus allows and perhaps promotes the activation of the latter in a stable form

## 7.1 Chloroform Pretreatment:

1. 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 minutes to separate two layers. Carefully pipette top layer containing pretreated plasma in a separate Eppendorf tube.
2. 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 minutes.
3. To each Eppendorf tube, add 10 µL of Coagulation Activator Solution/PK Activator solution and mix well by gentle tapping the tube. Incubate at 37°C for additional 5 minutes (or on ice for 45 minutes). Transfer this entire solution to a microplate well. Bring the final volume in each well to 50 µL with Assay Buffer XXXV/PK Assay Buffer.
4. Optional: Centrifuge the tube at 3000 x *g* for 5 minutes and remove the solution from activator. Load this solution on a microplate well. Bring the final volume in each well to 50 µL with Assay Buffer XXXV/PK Assay Buffer.

# 8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

## 8.1 Positive Control:

Use 1-20 µL of reconstituted Native Human PK/human PK enzyme solution in a separate well with and without 1 µL of PKSI Inhibitor. Incubate at room temperature for 10 minutes. Bring the final volume in each well to 50 µL with Assay Buffer XXXV/PK Assay Buffer.

## 8.2 Reaction mix:

Prepare 50 µL of PK Assay Mix per well. Prepare a master mix to ensure consistency.

| Component | Reaction Mix (µL) |
|---|---|
| Assay Buffer XXXV/PK Assay Buffer | 49 |
| Kallikrein Substrate/PK Substrate | 1 |

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 Standard I/*p*NA Standards.

## 8.3 Measurement:

1. For pNA Standard I/*p*NA Standards, measure the absorbance at 405 nm (OD405) in end point.
2. For PK Enzyme, Inhibitor Control and Plasma containing Samples, measure the absorbance at 405 nm (OD405) in kinetic mode for 0.5-1 hour at 37 °C.

ΔNote: It is recommended to run at least 3-5 different amounts of Plasma samples to get accurate measurements of plasma PK activity.

ΔNote: If plasma PK activity is low, higher amounts of chloroform-treated plasma can be activated with equal volume of Coagulation Activator Solution/PK activator and used in the assay.

## 9. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. ***p*NA 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.
2. **Plasma Samples:** Use the linear region of kinetic progress curves to obtain slopes for all Activated Plasma containing reactions and Inhibitor Control.
3. Choose two time points (t1 & t2) in the linear range of the plot and obtain the corresponding values for the absorbance.
4. Calculate ΔOD405/Δt for each Activated Plasma Sample and corresponding Inhibitor Control.
5. Subtract ΔOD405/Δt of the Inhibitor Control from Activated Plasma Sample and obtain corresponding (B, ΔOD405/min).
6. Using this value, calculate Plasma PK activity using following equation:

$$\text{PK Activity (mU/mLor U/L)} = \frac{\text{B x 1000}}{\text{A x X}}$$

Where:

B = Plasma PK Activity as calculated (ΔOD405/min).

X = µL of Plasma Sample used in the assay.

A= Slope of the pNA standard curve (ΔOD405/nmol).

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

## 10. Typical Data

Data provided **for demonstration purposes only**.

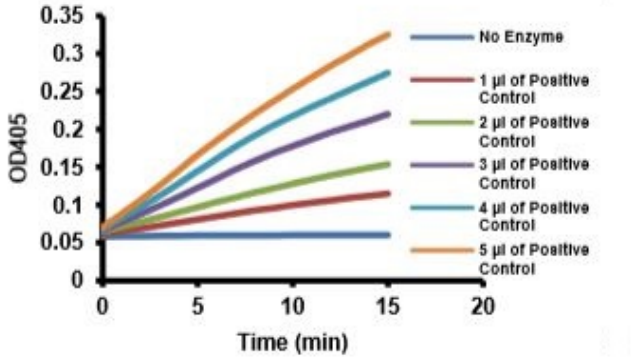


**Figure 1**. Kinetic progressive curves for different amounts of PK Enzyme.

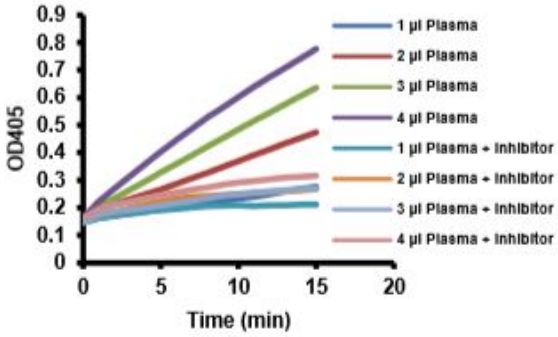


**Figure 2**. Kinetic progressive curves for different amounts of Activated Plasma Samples.

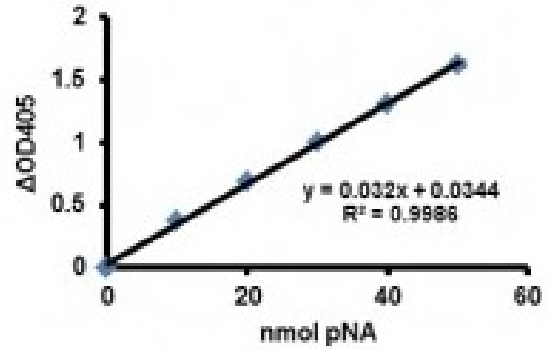


**Figure 3.** Standard curve for *p*NA (n = 3).

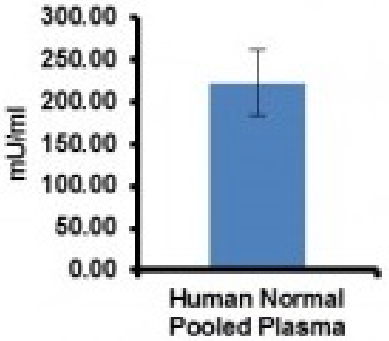


**Figure 4.** Standard curve for *p*NA (n = 3) (Figure 3) was used to estimate PK activity in Normal Pooled Human Plasma (n = 3).

# 11. Notes

## Technical Support



## For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)