

Version 2 Last updated 21 June 2019

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.

Table of Contents

1. Overview	3
2. Materials Supplied and Storage	4
3. Materials Required, Not Supplied	5
4. General guidelines, precautions, and troubleshooting	6
5. Reagent Preparation	7
6. Standard Preparation	8
7. Sample Preparation	9
8. Assay Procedure	10
9. Data Analysis	12
10. Typical Data	13
11. Notes	15

1. Overview

Plasma Kallikrein Assay Kit (ab241042) 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.

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)
PK Assay Buffer	25 mL	-20°C	4°C or -20°C
PK Activator	1 mL	-20°C	RT
PK Substrate	0.1 mL	-20°C	-20°C
Human PK	1 vial	-20°C	-20°C
PKSI Inhibitor	0.1 mL	-20°C	-20°C
pNA Standard (0.1 M)	20 µL	-20°C	-20°C

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 PK Assay Buffer

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

5.2 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 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 Human PK

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

5.6 pNA 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 μ L 0.1 M ρ NA Standard into 95 μ L PK Assay Buffer to prepare 5 mM ρ NA.
 2. Add 0, 2, 4, 6, 8, 10 μ L of 5 mM ρ NA standard into each well.
 3. Adjust volume to 100 μ L/well with PK Assay Buffer to generate 0, 10, 20, 30, 40, 50 nmol/well of ρ NA standard.

Standard #	5 mM ρ NA Standard (μ L)	PK Assay Buffer (μ L)	Final volume standard in well (μ L)	ρ 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 $\times g$ 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 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 PK Assay Buffer.
4. Optional: Centrifuge the tube at 3000 $\times g$ 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 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 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 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)
PK Assay Buffer	49
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 $p\text{NA}$ Standards.

8.3 Measurement:

1. For $p\text{NA}$ 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.

Δ Note: 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 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. **pNA Standard Curve:** Obtain change in the absorbance ΔOD_{405} by subtracting absorbance of the 0 Standard Controls from those containing all standards. Plot the ΔOD_{405} against nmol of pNA. The plot should be linear; determine the slope A ($\Delta OD_{405}/nmol$) 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 $\Delta OD_{405}/\Delta t$ for each Activated Plasma Sample and corresponding Inhibitor Control.
5. Subtract $\Delta OD_{405}/\Delta t$ of the Inhibitor Control from Activated Plasma Sample and obtain corresponding (B, $\Delta OD_{405}/min$).
6. Using this value, calculate Plasma PK activity using following equation:

$$PK \text{ Activity (mU/mL or U/L)} = \frac{B \times 1000}{A \times X}$$

Where:

B = Plasma PK Activity as calculated ($\Delta OD_{405}/min$).

X = μL of Plasma Sample used in the assay.

A = Slope of the pNA standard curve ($\Delta OD_{405}/nmol$).

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

10. Typical Data

Data provided for demonstration purposes only.

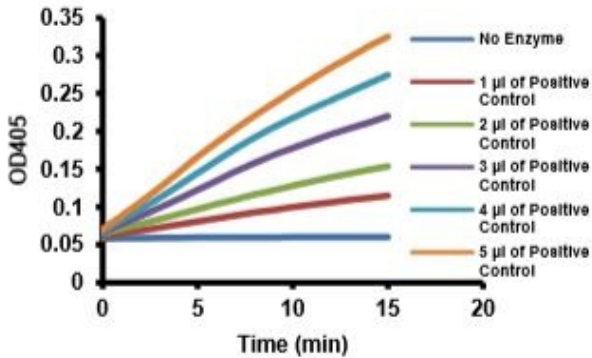


Figure 1. Kinetic progressive curves for different amounts of PK Enzyme.

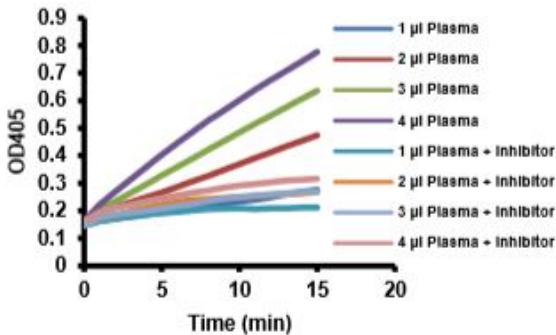


Figure 2. Kinetic progressive curves for different amounts of Activated Plasma Samples.

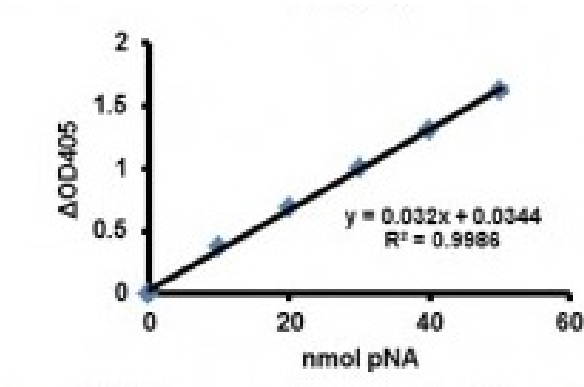


Figure 3. Standard curve for pNA (n = 3).

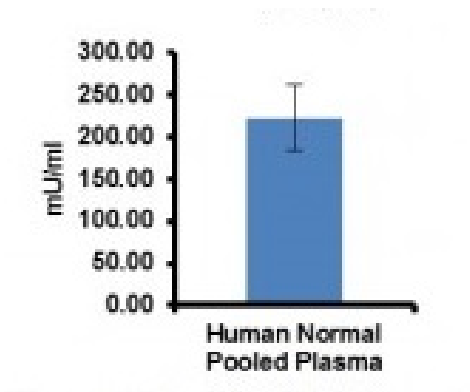


Figure 4. Standard curve for pNA (n = 3) (Figure 3) was used to estimate PK activity in Normal Pooled Human Plasma (n = 3).

11. Notes

Technical Support

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