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ab273312 Pyridoxal 5'-phosphate (Vit B6) Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273312
(use https://www.abcam.cn/ab273312 for china, or
https://www.abcam.co.jp/ab273312 for Japan)

For the estimation of Pyridoxal 5'-phosphate (Vit B6) concentration in biological samples and beverages.

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

Table of Contents

1. Overview

Pyridoxal 5'-phosphate (Vit B6) Assay Kit (Fluorometric) (ab273312) provides a quick, specific and easy method for measuring Pyridoxal 5'-phosphate (PLP) concentrations in a wide variety of samples.

In this assay, PLP reacts with a PLP-dependent enzyme thereby converting the substrate into an intermediate, which will further react with a probe to produce a strong fluorometric signal (Ex/Em = 535/587 nm). The kit is simple, easy to use, sensitive and high-throughput adaptable. It can detect as low as 0.2 pmol/well of PLP in biological samples.

2. Protocol Summary

Prepare samples as directed.

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Prepare all reagents as directed.

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Prepare standards.

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Add Standard, Sample, and Blanks (PLP Assay Buffer) to appropriate wells and adjust volume to 50 µL.

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Add Sample Mix to Standard, Blanks and Sample wells.

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Mix and incubate at 25°C for 30 mins protected from light.

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Add Detection Mix to Standard, Blanks and Sample wells.

⇩

Immediately measure fluorescence in kinetic mode
for 30 mins every 30 secs.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 6 months from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
PLP Assay Buffer	25 mL	-20°C	+4°C or -20°C
PLP Substrate (Lyophilized)	1 vial	-20°C	-20°C
PLP Enzyme Mix (Lyophilized)	1 vial	-20°C	-80°C
PLP Developer/PLP Developer 1 (Lyophilized)	1 vial	-20°C	-20°C
Developer Solution V/PLP Developer 2 (Lyophilized)	1 vial	-20°C	-20°C
OxiRed Probe/PLP Probe	200 µL	-20°C	+4°C or -20°C
PLP Standard (Lyophilized)	1 vial	-20°C	-20°C

7. Materials Required, Not Supplied

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

- Multi-well spectrophotometer capable of measuring fluorescence at Ex/Em= 535/587 nm
- Black 96-well plate with flat bottom
- Dounce homogenizer

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 **PLP Assay Buffer:**
Ready to use as supplied. Store at 4°C or -20°C. Bring to room temperature before use.

9.2 **PLP Substrate (Lyophilized):**
Reconstitute with 220 µL PLP Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze/thaw cycles. Use within two months.

9.3 **PLP Enzyme Mix (Lyophilized):**
Reconstitute with 220 µL PLP Assay Buffer. Aliquot and store at -80°C. Keep on ice while in use. Avoid freeze/thaw cycles. Use within two months.

9.4 **PLP Developer/PLP Developer 1 (Lyophilized):**
Reconstitute with 220 µL PLP Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze/thaw cycles. Use within two months.

9.5 **Developer Solution V/PLP Developer 2 (Lyophilized):**
Reconstitute with 220 µL PLP Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze/thaw cycles. Use within two months.

9.6 **OxiRed Probe/PLP Probe:**
Ready to use as supplied. Warm to room temperature before use. Store at 4°C or -20°C, away from light.

9.7 **PLP Standard (Lyophilized):**
Reconstitute with 200 µL of dH_2O to make stock 10 µM PLP Standard solution. Store the stock 10 µM PLP Standard solution at -20°C, away from light.

10. Sample Preparation

Tissues Samples:

10.1 Rapidly homogenize tissue (~10 mg) in 100 µL ice cold PLP Assay Buffer with Dounce Tissue Homogenizer and keep on ice for 10 minutes.

All Other Samples:

10.2 Centrifuge the Sample at 13,000 *x g* and 4°C for 10 minutes to remove the precipitate from the liquid.

10.3 Collect the supernatant and add 200-500 µL of the supernatant into a 10 kDa Spin Column.

10.4 Centrifuge the Sample at 13,000 *x g* and 4°C for 20 minutes and collect the filtrate for the assay.

Δ Note: PLP varies over a wide range for different Samples. For Unknown Samples, we recommend performing a pilot experiment with several Sample dilutions to ensure that the readings are within the Standard Curve range. For normal human serum, average PLP concentration ranges around 10-200 nM.

11. Standard Curve

11.1 Prepare a working 100 nM solution of PLP Standard by diluting 10 µL of the stock 10 µM PLP Standard with 990 µL of dH_2O.

11.2 Add 0, 2, 4, 6, 8 and 10 µL of the working 100 nM solution of PLP Standard into a series of wells in a black 96-well plate to obtain 0, 200, 400, 600, 800 and 1000 fmol/well.

11.3 Adjust the volume of each well to 50 µL with PLP Assay Buffer

Standard #	100 nm PLP Standard (µL)	PLP Assay Buffer (µL)	PLP Standard (fmol/well)
1	10	40	1000
2	8	42	800
3	6	44	600
4	4	46	400
5	2	48	200
6	0	50	0

12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

Δ Note: Major matrix effect can arise from Serum Sample. We do not recommend using more than 10 µL Human Serum Sample per well of a 96-well plate for the assay.

12.1 For Sample (S), add 2 -50 µL of the pretreated, filtered Sample(s).
12.2 Adjust the volume of Sample to 50 µL/well with PLP Assay Buffer. Mix well.
12.3 For Blank, add 50 µL of PLP Assay Buffer into another well.
12.4 **Enzyme Mix:** Prepare enough reagents for the number of assays to be performed. PLP Enzyme Mix is a suspension. Thus, vortex the tube every time before adding to the Sample Mix. For each well, prepare 20 µL of Sample Mix. Mix well.

Component	Reaction Mix (µl)
PLP Enzyme Mix	2
PLP Assay Buffer	18

12.5 Add 20 µL of the Sample Mix to each well containing Standards, Blank and Sample(s).
12.6 Mix well and incubate the plate for 30 minutes at 25°C. Avoid light.
12.7 **Detection Mix:** Prepare enough reagents for the number of assays to be performed. Prepare 5X dilution of the OxiRed Probe/PLP Probe with PLP Assay Buffer. (i.e. add 5 µL of OxiRed Probe/PLP Probe into 20 µL PLP Assay Buffer). For each well, prepare 30 µL Detection Mix. Mix well.

Component	Detection Mix (µl)
PLP Assay Buffer	22
PLP Substrate	2
PLP Developer/PLP Developer 1	2
Developer Solution V/PLP Developer 2	2
Diluted OxiRed Probe/PLP Probe	2

12.8 Add 30 µL of Detection Mix to each well containing Blank, Standard and Sample(s). Mix well.

12.9 Immediately, measure fluorescence (Ex/Em = 535/587 nm) in a plate reader in kinetic mode for 30 minutes every 30 seconds.

13. Calculations

13.1 Subtract 0 Standard readings from all Standard readings.
13.2 Plot the RFU vs time.
13.3 Obtain the initial slope (RFU/min).
13.4 Plot the initial slope against the PLP amounts in each well and obtain the PLP Standard Curve.
13.5 Obtain the corrected Sample (RFU_{CS}) readings by subtracting Blank (RFU_{BL}) reading from Sample (RFU_S) readings, ($RFU_{CS} = RFU_S - RFU_{BL}$).
13.6 Plot $RFUC_S$ vs time and use the linear portion of the curve to determine the slope of the kinetic curve.
13.7 Check slope against the PLP Standard Curve to obtain the amount of PLP in the wells (B).

$$Concentration\ of\ PLP\ in\ fluid\ sample = \frac{B}{V} x\ D = fmol/\mu L = nM$$

$$Concentration\ of\ PLP\ in\ tissue\ sample = \frac{B}{(V\ x\ P)} x\ D = fmol/\mu g = pmol/mg$$

B = Amount of PLP calculated from the Standard Curve (in fmol)
V = Volume of sample added to the well (in µl)
D = Sample dilution factor (if applicable, D=1 for undiluted samples)
P = Concentration of protein (in µg/µL)

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

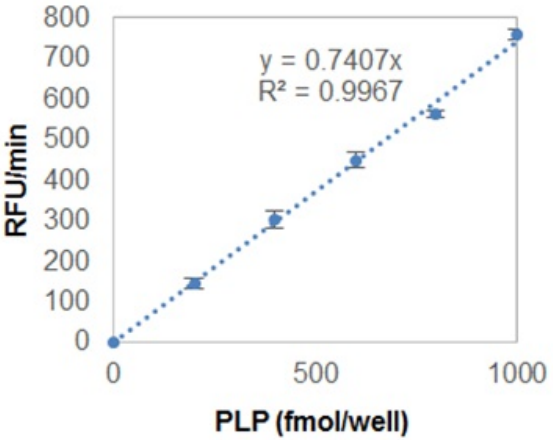

Figure 1. PLP standard curve.

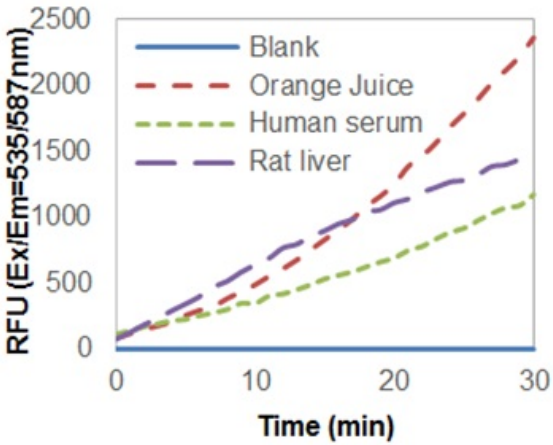

Figure 2. Corrected kinetic curve for Blank, orange juice, human serum and rat liver lysate.

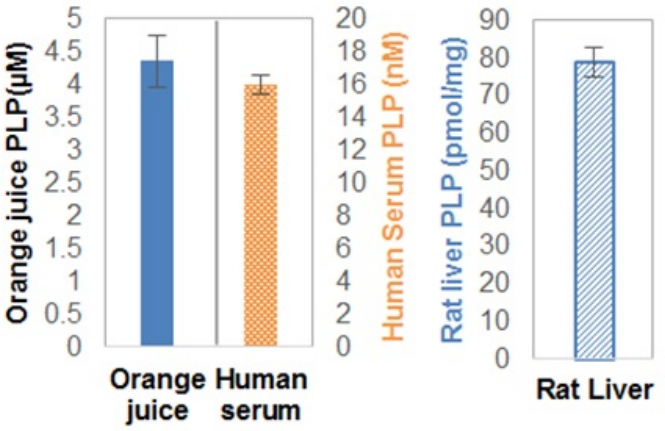

Figure 3. Estimation of PLP in orange juice (5 µL of 100X dilution), human serum (4 µL) and rat liver lysate (5 µL of 10X dilution). PLP concentrations were 4.36± 0.40 µM in orange juice, 15.98 ± 0.58 nM in human serum and 79.0 ± 4.0 pmol/mg in rat liver lysate samples. Assays were performed following the kit protocol.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. 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)