Product Manual

Pyridoxial-5'-phosphate (PLP) Assay Kit

Catalog Number

MET-5216 **200 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
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Introduction

Pyridoxial-5'-phosphate (PLP), the active form of Vitamin B6, is an enzymatic cofactor. PLP is covalently bound to the amino group of a lysine in the active site of enzymes. There are a wide variety of enzymes that require PLP for their activities. These consist of enzymes involved in amino acid metabolism reactions including transamination, decarboxylation, deamination. PLP is available from numerous food sources or through supplements. Vitamin B6 deficiency can lead to neurological, dermatological, and hematological complications.

Cell Biolabs' PLP Assay Kit is a simple assay for measuring PLP levels in biological samples including serum, plasma, and cell or tissue lysates. The kit has a detection sensitivity limit of 3.9 ng/mL PLP. Each kit provides sufficient reagents to perform up to 200 assays*, including PLP standards, assay controls and unknown samples.

Note: Each sample replicate requires 2 assays, one treated with D-Phenylglycine Amino Transferase (+D-PhgAT) and one without (-D-PhgAT). PLP levels are calculated from the difference in OD readings from the 2 wells.

Assay Principle

The PLP assay has two steps. First, PLP present in unknown samples or PLP Standards is allowed to reconstitute and activate the apo-D-PhgAT, and form active D-PhgAT. Second, the enzymatic activity of D-PhgAT is determined by using D-4-hydroxyphenylglycine (OHPhg) as an amino donor substrate. OHPhg is converted to 4-hydroxybenzoylformate (OHBZF) (Figure 1) and the UV absorbance of OHBZF is read at 334 nm. The rate of the transamination reaction catalyzed by the reconstituted D-PhgAT is directly proportional to the amount of PLP in the sample. The content of PLP in the unknown samples is determined by comparison with a predetermined PLP standard curve.

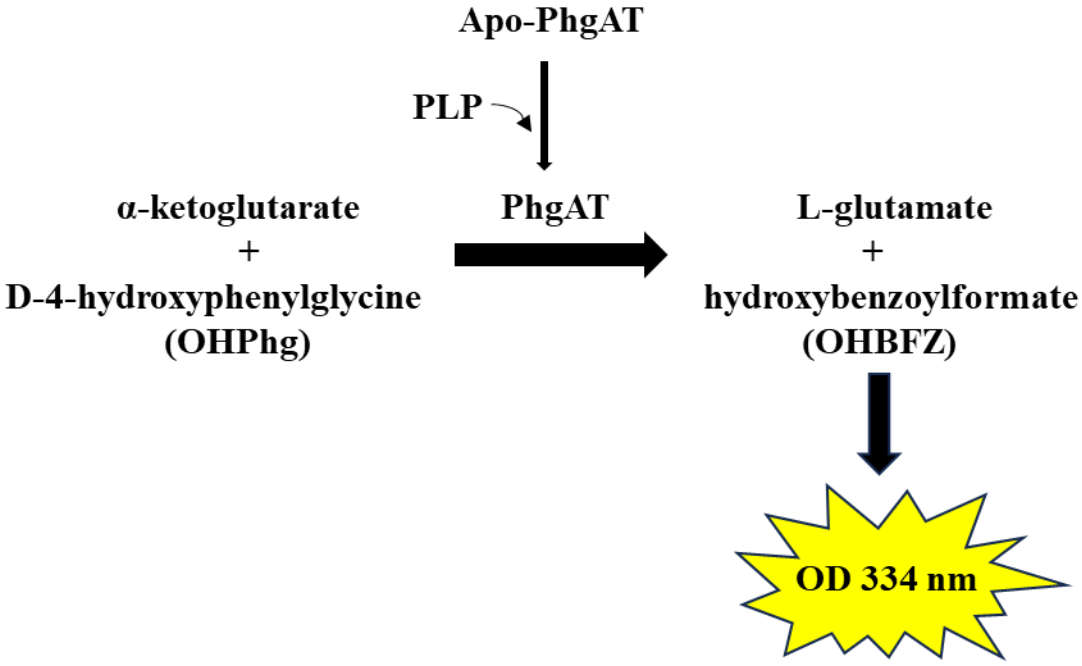

Figure 1. Assay Principle.

Related Products

1. MET-5207: Lipoic Acid ELISA Kit
2. MET-5208: Magnesium Assay Kit
3. MET-5014: NAD+/NADH Assay Kit (Colorimetric)
4. MET-5138: Zinc Assay Kit (Colorimetric)
5. MET-5213: PQQ Assay Kit

Kit Components (shipped on dry ice)

1. D-PhgAT Activation Buffer (Part No. 52161B): Two 2 mL vial
2. Apo-D-PhgAT (Part No. 52163D): One 1 mL vial
3. D-PhgAT Substrate Solution (Part No. 52164B): One 20 mL bottle
4. PLP Standard (Part No. 52164D): One 100 µL vial at 25 µg/mL

Materials Not Supplied

1. Distilled or deionized water
2. Standard 96-well clear microtiter plate
3. Spectrophotometric microplate reader capable of reading UV absorbance at 334 nm

Storage

Upon receipt, store the D-PhgAT Activation Buffer and D-PhgAT Substrate Solution at 4ºC. Store all remaining components at -80ºC. Avoid multiple freeze/thaw cycles.

Preparation of Reagents

Note: All reagents must be brought to room temperature prior to use.

- Activation Mix: Prepare Activation Mix by adding Apo-D-PhgAT, D-PhgAT Activation Buffer and deionized water at a ratio of 1:4:5. For example, for 10 assays, add 50 µL of Apo-D-PhgAT and 200 µL of Activation Buffer to 250 µL of deionized water.

  Note: Scale the described example appropriately and prepare only enough for immediate use.

- Control Mix: Prepare Control Mix by adding D-PhgAT Activation Buffer and deionized water at a ratio of 2:3. For example, for 10 assays, add 200 µL of Activation Buffer to 300 µL of deionized water.

  Note: Scale the described example appropriately and prepare only enough for immediate use.

Preparation of Samples

Notes: All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with unknown samples.

- Cell lysates: Resuspend cells in PBS. Homogenize or sonicate the cells on ice. Centrifuge 10,000 x g for 10 minutes at 4°C to remove debris. Collect the supernatant. The supernatant may be assayed undiluted or diluted as necessary into PBS.

- Tissue lysates: Sonicate or homogenize tissue sample in PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Collect the supernatant. The supernatant may be assayed directly or diluted as necessary into PBS.
- Serum, Plasma, or Urine: Samples should be centrifuged for 10 min at 10,000 rpm to remove insoluble particles. Samples can be deproteinated by running it through a 3 kDa MWCO centrifugal filter unit collecting the flow through. Dilute the supernatant as necessary with PBS just prior to performing the assay.

Preparation of Standard Curve

Freshly prepare a dilution series of PLP Standard in the concentration range of 250 ng/mL – 0 ng/mL by diluting the 25 µg/mL PLP standard solution in deionized water (Table 1).

Standard Tubes	25 µg/mL PLP Standard (µL)	Deionized Water (µL)	PLP (ng/mL)
1	5	495	250
2	250 of Tube #1	250	125
3	250 of Tube #2	250	62.5
4	250 of Tube #3	250	31.3
5	250 of Tube #4	250	15.6
6	250 of Tube #5	250	7.8
7	250 of Tube #6	250	3.9
8	0	250	0

Table 1. Preparation of PLP Standards

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

   Note: Each sample replicate requires two paired wells, one to be treated with PhgAT (Activation Mix) and one without PhgAT (Control Mix) to measure endogenous sample background.
2. Add 50 µL of each sample (PLP standard or unknown) into wells of a 96-well plate.
3. Add 50 µL of Activation Mix to the standards and to one half of the paired sample wells and mix the well contents thoroughly.
4. Add 50 µL of Control Mix to the other half of the paired sample wells and mix thoroughly.
5. Incubate at room temperature for 30 minutes.
6. Add 100 µL of D-PhgAT Substrate Solution to each well and mix thoroughly.
7. Incubate at room temperature for 30 minutes.

   Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.
8. Read UV absorbance of each well on a microplate reader using 334 nm.

Example of Results

The following figures demonstrate typical PLP Assay results. One should use the data below for reference only. This data should not be used to interpret actual results.

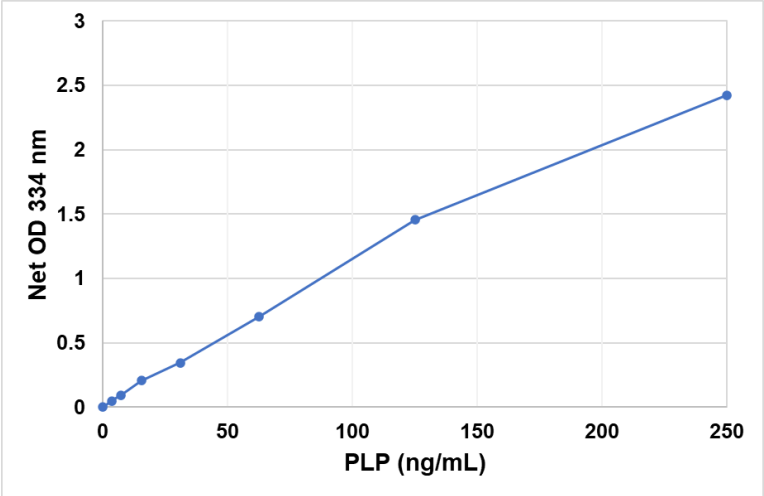

Figure 2. PLP Standard Curve.

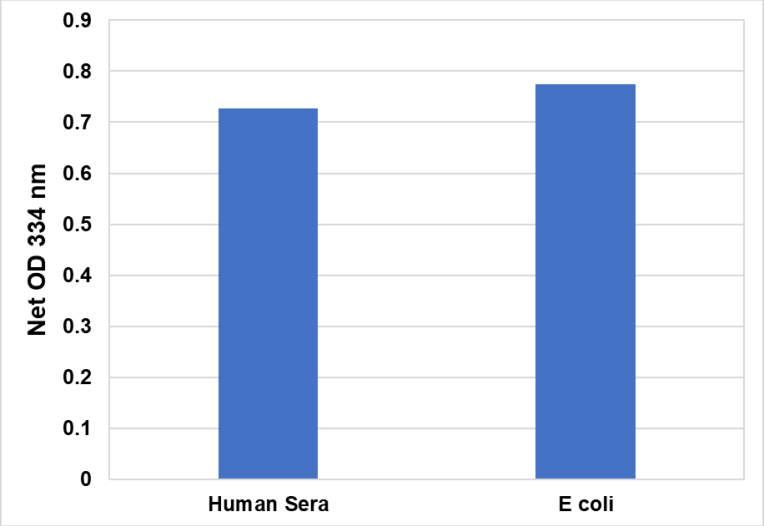

Figure 3. Detection of PLP in Human Serum and *E. coli*. Undiluted human serum and *E. coli* lysate were assayed according to the kit protocol.

Calculation of Results.

1. Determine the average absorbance values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 2).
4. Subtract the sample well values without PhgAT (-PhgAT) from the sample well values containing enzyme (+PhgAT) to obtain the difference. The absorbance difference is due to the enzyme PhgAT activity:

$$\Delta A = A_{(+PhgAT)} - A_{(-PhgAT)}$$

5. Compare the change in absorbance ΔA of each sample to the standard curve to determine and extrapolate the quantity of PLP present in the sample. Only use values within the range of the standard curve.

References

1. Eliot AC, and Kirsch JF (2004) *Annu Rev Biochem* **73**: 383-415
2. Jomrit J, Isarangkul D, Summpunn P, and Wiyakrutta S (2018) *Enz Microb Tech* **117**: 64-71
3. Chantarasiri A, Meevootisom V, Isarangkul D, and Wiyakrutta S (2012) J Mol Micriobio Biotechnol **22**: 147-155

Warranty

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