Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Pyridoxal 5'-phosphate (Vit B_6) Fluorometric Assay Kit

07/19

(Catalog #K2009-100; 100 assays; Store at -20°C)

I. Introduction:

Pyridoxal 5'-phosphate (PLP), the active form of Vitamin B_6 is a cofactor in a variety of metabolic reactions including transamination, decarboxylation, racemization, etc. Vitamin B_6 comprises of pyridoxine, pyridoxamine and pyridoxal, which during metabolism is converted to the enzymatically active form namely PLP. Vitamin B_6 is necessary for the synthesis of neurotransmitters, hemoglobin in red blood cells, fat metabolism etc. Vitamin B_6 deficiency can lead to nervous disorders, peripheral neuropathy, anemia, seizures etc. BioVision's Pyridoxal 5'-phosphate (Vit B_6) Fluorometric Assay Kit provides a quick, specific and easy method for measuring 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.

Pyridoxal 5'-Phosphate —(PLP-dependent enzyme)→ Intermediate —(Probe)→ Fluorescence detection (Ex/Em = 535/587 nm)

II. Applications:

- Measurement of PLP in biological samples and beverages
- Analysis of Vitamin B_6 in beverage and effects of Vitamin B6 intake on serum

III. Sample Type:

- Biological fluids: serum, juices, etc.
- Mammalian tissue

IV. Kit Contents:

Components	K2009-100	Cap Code	Part Number
PLP Assay Buffer	25 ml	WM	K2009-100-1
PLP Substrate	1 vial	Orange	K2009-100-2
PLP Enzyme Mix	1 vial	Purple	K2009-100-3
PLP Developer 1	1 vial	Blue	K2009-100-4
PLP Developer 2	1 vial	Green	K2009-100-5
PLP Probe	200 µl	Red	K2009-100-6
PLP Standard	1 vial	Yellow	K2009-100-7

V. User Supplied Reagents and Equipment:

- 96-well black plate with flat bottom
- Multi-well spectrophotometer (plate reader)
- Dounce Homogenizer

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **PLP Assay Buffer:** Warm to room temperature (RT) before use. Store at 4ºC or -20ºC.
- **PLP Substrate, PLP Developer 1 & Developer 2:** Reconstitute each vial 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.
- **PLP Enzyme Mix:** 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.
- **PLP Probe:** Ready to use as supplied. Warm to RT before use. Store at 4°C or -20°C, away from light.
- **PLP Standard:** 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.

VII. Pyridoxal 5'-phosphate (Vit B_6) Assay Protocol:

1. **Sample Preparation: For Tissue Samples:** Rapidly homogenize tissue (~10 mg) in 100 µl ice cold PLP Assay Buffer with Dounce Tissue Homogenizer (BioVision Cat# 1998), and keep on ice for 10 min. **For all Samples:** Centrifuge the Sample at 13,000 x *g* and 4°C for 10 min to remove the precipitate from the liquid. Collect the supernatant and add 200-500 µl of the supernatant into a 10 kDa Spin Column (BioVision Cat# 1997). Centrifuge the Sample at 13,000 *x g* and 4°C for 20 min and collect the filtrate for the assay. In a 96-well black plate, add 2-50 µl of the pretreated, filtered Sample(s) labeled as **Sample**. Adjust the Sample volume to 50 µl with PLP Assay Buffer. Add 50 µl of PLP Assay Buffer into another well labeled as **Blank**.

   Notes:

   a. 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.

   b. 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.

2. **Standard Curve Preparation:** Prepare a working 100 nM PLP Standard solution by adding 10 µl of the stock 10 µM PLP Standard to 990 µl of dH_2O. Add 0, 2, 4, 6, 8, 10 µl of the working 100 nM PLP Standard into a series of wells generating 0, 200, 400, 600, 800, 1000 fmol of PLP/well. Adjust the volume to 50 µl/well with PLP Assay buffer.
3. **Enzyme Mix:** Mix 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 Sample Mix containing:

	Sample Mix
PLP Enzyme Mix	2 µl
PLP Assay Buffer	18 µl

Mix and add 20 µl of the Sample Mix to each well containing Standards, Blank and Sample(s). Mix well and incubate the plate for 30 min at 25°C. Avoid light.

4. **Detection Mix:** Mix enough reagents for the number of assays to be performed. Prepare 5X dilution of the PLP Probe with PLP Assay Buffer. (i.e. add 5 µl of PLP Probe into 20 µl PLP Assay Buffer). For each well, prepare 30 µl Detection Mix containing:

	Detection Mix
PLP Assay Buffer	22 µl
PLP Substrate	2 µl
PLP Developer 1	2 µl
PLP Developer 2	2 µl
Diluted PLP Probe	2 µl

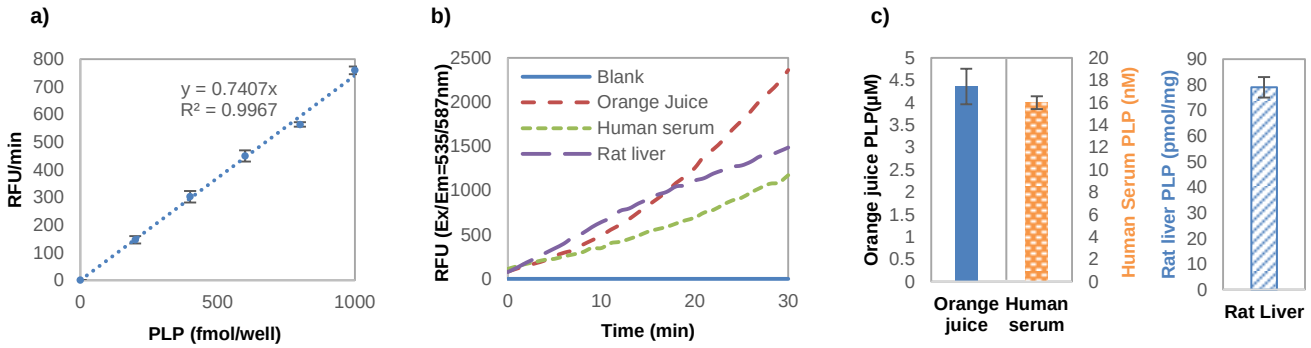
Mix and add 30 µl of the Detection Mix to each well containing Blank, Standard and Sample(s). Mix well.

5. **Measurement:** Immediately, measure fluorescence (Ex/Em = 535/587 nm) in a plate reader in kinetic mode for 30 min every 30 sec.
6. **Calculation:** Subtract 0 Standard readings from all Standard readings. Plot the RFU vs time. Obtain the initial slope (RFU/min). Plot the initial slope against the PLP amounts in each well and obtain the PLP Standard Curve. Obtain the corrected Sample (RFU_{CS}) readings by subtracting Blank (RFU_{BL}) reading from Sample (RFU_S) readings, **($RFU_{CS} = RFU_S - RFU_{BL}$)**. Plot RFU_{CS} vs time and use the linear portion of the curve to determine the slope of the kinetic curve. Check slope against the PLP Standard Curve to obtain the amount of PLP in the wells (B).

Concentration of PLP in fluid sample = $\frac{B}{V} \times D$ = fmol/µl = nM

Concentration of PLP in tissue sample = $\frac{B}{V \times P} \times D$ = fmol/ µg = pmol/mg

Where: B is the amount of PLP calculated from the Standard Curve (in fmol)
V is the volume of Sample added to the well (in µl)
D is the Sample dilution factor (if applicable, D=1 for Undiluted Samples)
P is the concentration of protein (in µg/µl)



Figures: (a) PLP Standard Curve. (b) Corrected kinetic curve for Blank, Orange Juice, Human serum and Rat liver lysate. (c) 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.

VIII. Related Products:

Ascorbic acid Assay Kit (BV Cat# K661/K671)
Total Antioxidant capacity Assay Kit (BV Cat# K274)
Phenolic Compounds Assay kit (BV Cat# K527)
Vitamin D3 (human) ELISA kit (BV Cat# K4806)

FOR RESEARCH USE ONLY! Not to be used on humans.