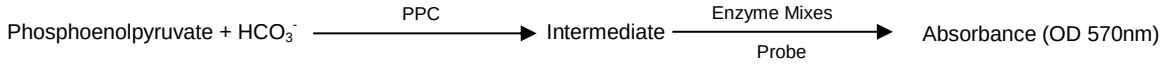
Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Phosphoenolpyruvate Carboxylase Activity Assay Kit (Colorimetric) *rev.12/18*

(Catalog #K443-100; 100 assays; Store at -20°C)

I. Introduction:

Phosphoenolpyruvate carboxylase (EC 4.1.1.31, PPC) is an enzyme found in plants and some types of bacteria. It catalyzes the formation of oxaloacetate from phosphoenolpyruvate by fixing one carbon in the forms of either carbon dioxide or bicarbonate. PPC participates in carbon fixation in C4 photosynthesis and Crassulacean acid metabolism (CAM) plants and acts as an anaplerotic enzyme, which replenishes TCA cycle intermediates, in bacteria and C3 plants. The participation of PPC in the carbon fixation cycle of C4 plants enable them to survive in high sunlight but dry environments. BioVision's Phosphoenolpyruvate Carboxylase Assay Kit provides a quick and easy method for the measurement of PPC activity in various samples. In this assay, Phosphoenolpyruvate Carboxylase is coupled with a set of enzymes that convert PEP and carbonate into a series of intermediates and hydrogen peroxide, which in turn, reacts with a probe and converter generating a colorimetric signal (OD 570 nm). The generated absorbance signal is directly proportional to the amount of active PPC present in the samples. This kit provides a simple, fast and high throughput adaptable assay to measure the activities of PPC as low as 0.2 mU.



II. Applications:

- Measurement of phosphoenolpyruvate carboxylase activities in plant or bacteria samples
- Analysis of carbon fixation pathway in plants
- Studies of the anaplerotic metabolic pathway in bacteria

III. Sample Type:

- Plant Tissue
- Bacterial Cells

IV. Kit Contents:

Components	K443-100	Cap Code	Part Number
PPC Assay Buffer	25 ml	WM	K443-100-1
PPC Substrate A	1 vial	Blue	K443-100-2
PPC Substrate B	220 µl	Brown	K443-100-3
PPC Probe (in DMSO)	200 µl	Red	K443-100-4
PPC Enzyme Mix	1 vial	Purple	K443-100-5
PPC Standard (100 mM)	100 µl	Yellow	K443-100-6
PPC Positive Control	1 vial	Orange	K443-100-7

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- For Plants tissue: Liquid nitrogen, Dounce Tissue Homogenizer (Cat. #1998)
- For Bacterial culture: Sonicator

VI. Storage Conditions and Reagent Preparation:

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

- **PPC Assay Buffer:** Warm to room temperature before use. Store at 4ºC or -20ºC.
- **PPC Substrate A:** Reconstitute with 220 µl PPC Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use.
- **PPC Substrate B:** Ready to use as supplied. Warm to room temperature before use and vortex to dissolve completely. Keep on ice while in use. Store at -20°C.
- **PPC Probe (in DMSO):** Ready to use as supplied. Warm to room temperature before use. Store at -20°C. Avoid from light.
- **PPC Enzyme Mix:** Reconstitute with 220 µl PPC Assay Buffer. Pipette up and down to dissolve. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Keep on ice while in use.
- **PPC Assay Standard (100 mM):** Ready to use as supplied. Keep on ice while in use. Store at -20°C.
- **PPC Positive Control:** Reconstitute with 100 µl PPC Assay Buffer. Pipette up and down to dissolve. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Keep on ice while in use.

VII. PPC Assay Protocol:

1. **Sample Preparation: For plant tissue samples:** Grind tissue samples in liquid nitrogen. Rapidly homogenize tissue (~25 mg) (BioVision: Cat #1998) with 100 µl ice cold PPC Assay Buffer, and keep on ice for 10 min. Centrifuge at 16,000 x g for 10 min at 4°C to remove cell debris and collect the supernatant. **For bacterial samples:** Collect bacteria culture (cell count ~6 X 10^9). Centrifuge at 4,000 x g for 5 min to collect the cell pellet. Resuspend in 500 µl ice cold PPC Assay Buffer containing protease inhibitors (BioVision: Cat # K271). Lyse the bacteria with a sonicator, using short pulses and more cycles. **For all samples**: Since small molecules can interfere with the PPC activity, ultrafiltrate the samples with 10 kDa Spin Columns (BioVision: Cat#1997). Wash the retentate with PPC Assay Buffer containing protease inhibitors three times. Collect the retentate and bring its volume back to the original sample volume. Add 2-50 µl of samples into a clear plate and bring the volume to 50 µl with PPC Assay Buffer. For sample background control, add the

same amount of samples in parallel wells. **For positive control:** Add 2-10 µl of the reconstituted PPC Positive Control into a clear plate and bring the volume to 50 µl with PPC Assay Buffer.

Notes: For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

2. **Standard Curve Preparation:**

   Prepare 1 mM (1 nmol/ul) PPC Assay Standard by adding 10 µl of the 100 mM standard to 990 µl of dH_2O. Mix well. Add 0, 2, 4, 6, 8, 10 µl of the 1 mM standards into a 96-well plate to generate 0, 2, 4, 6, 8, 10 nmol/well. Bring the volume to 50 µl with the PPC Assay buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

	Reaction Mix	Background Mix
PPC Assay Buffer	42 µl	44 µl
PPC Substrate A	2 µl	----
PPC Substrate B	2 µl	2 µl
PPC Probe	2 µl	2 µl
PPC Enzyme Mix	2 µl	2 µl

   Mix and add 50 µl of the Reaction Mix to each well containing the Standard Curve, test samples and positive control. Add 50 µl of Background mix to the sample background control.

4. **Measurement:** Measure absorbance (OD 570 nm) immediately in a microplate reader in kinetic mode for 30-45 min at 30 °C. We recommend reading the plate every one minute or longer interval as desired.

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the PPC Assay Standard Curve. If sample background control reading is significant, subtract the background control reading from its paired sample reading. Apply Sample ΔOD ($OD_2 – OD_1$) to PPC Assay Standard Curve to get B nmol of product generated during the reaction time ($\Delta t = t_2 - t_1$).

   Note: A slow kinetic response in the reactions (Time lag) could be observed. Do not use the first 20 minutes of the reactions for the estimation of PPC activity taking OD.

Sample Phosphoenolpyruvate Carboxylase Activity = B/(Δt X V) x D = nmol/min/ml = mU/ml

Where: **B** = Standard amount from Standard Curve (nmol)
Δt = reaction time (min)
V = sample volume added into the reaction well (ml)
D = Dilution Factor

The specific activity in biological samples can be expressed as mU/µg of protein.

Unit Definition: One unit of phosphoenolpyruvate carboxylase is the amount of enzyme that generates 1.0 µmol of Oxaloacetate per min at pH 7.2 at 30°C.

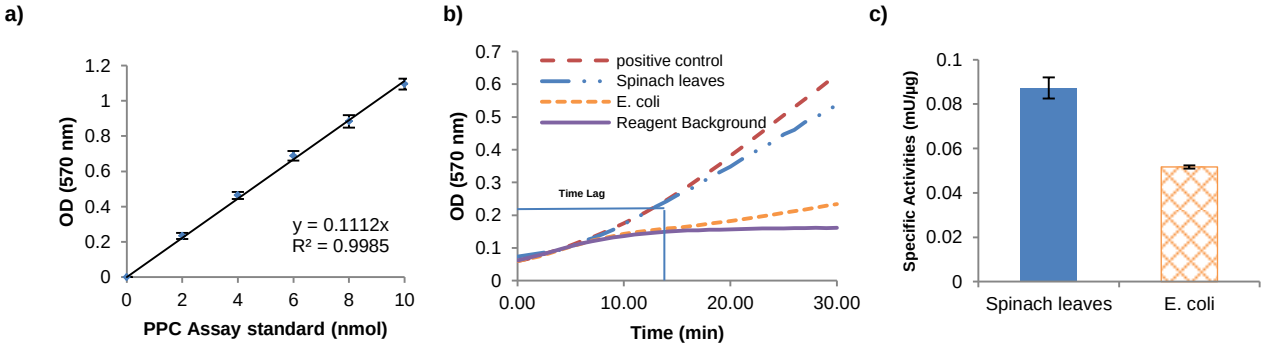


Figure: (a) PPC Assay Standard curve; (b) Phosphoenolpyruvate Carboxylase activities in Spinach leaf extract (8 µg protein), *E. coli* lysate (11 µg protein) & PPC positive control; (c) Specific PPC activities in Spinach leaves and *E. coli* cell lysate. Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

PEP Colorimetric/Fluorometric Assay Kit (K365)
PEPCK-C Blocking Peptide (3949BP)
Oxaloacetate Colorimetric/Fluorometric Assay Kit (K753)
Pyruvate Colorimetric/Fluorometric Assay Kit (K607)
Fumarase Activity Colorimetric Assay Kit (K677)
Glycogen Colorimetric/Fluorometric Assay Kit (K646)
Glycogen Colorimetric Assay Kit II (K648)
Magnesium Colorimetric Assay Kit (K385)
Aspartate Aminotransferase Activity Colorimetric Assay Kit (K753)
Alanine Aminotransferase Activity Assay kit (K752)

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