TECHNICAL MANUAL

Phosphoenolpyruvate Carboxylase (PEPC) Activity Assay Kit

- **SKU CODE:** MAES0280
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add sample to wells
- Add working solution and measure initial absorbance
- Incubate at 37°C and measure final absorbance
- Calculate PEPC activity

2. Intended use

This kit can be used to measure phosphoenolpyruvate carboxylase (PEPC) activity in plant tissue samples.

3. Detection principle

Phosphoenolpyruvate carboxylase (PEPC) catalyzes phosphoenolpyruvate (PEP) to produce oxaloacetic acid, which further reacts with NADH. NADH has maximum absorption at 340 nm. The activity of PEPC can be calculated by measuring the change in absorbance value at 340 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	13 mL × 1 vial	25 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 2	Substrate	Powder × 2 vials	Powder × 4 vials	2-8°C, 12 months, shading light
	UV Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (320-360 nm, optimum wavelength: 340 nm), Incubator (37°C)

Reagents:

PBS (0.01 M, pH 7.4)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of working solution::** Dissolve one vial of substrate with 6 mL of buffer solution, mix well to dissolve completely. Store at 2-8°C for 3 days protected from light.

7. Sample preparation

1. **Tissue sample preparation::** 1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg). 2. Wash tissue in cold PBS (0.01 M, pH 7.4). 3. Homogenize 20 mg tissue in 180 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C. 4. Centrifuge at 10000×g for 10 minutes at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. 5. Meanwhile, determine the protein concentration of supernatant (MAES0126).
2. **Dilution of sample::** The recommended dilution factor for different samples is as follows (for reference only): 10% Shanghai green tissue homogenate: 1 10% Sweet potato leaves tissue homogenate: 1 10% Crowndaisy chrysanthemum tissue homogenate: 1 10% Napa cabbage tissue homogenate: 1 Note: The diluent is PBS (0.01 M, pH 7.4). For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

It is recommended to use fresh plant samples for determination.

9. Operating steps

1. Sample well: Add 40 μL of sample to the wells.
2. Add 200 μL of working solution into each well. Mix fully and stand for 1 min at room temperature. Measure the OD values of each well at 340 nm with microplate reader. Record as A1.

3. Incubate at 37°C for 10 min, then measure the OD values of each well at 340 nm with microplate reader. Record as A2. ΔA340 = A1 - A2.

10. Calculation

Tissue sample:

Unit definition: The enzyme amount that consumes 1 μmol of NADH by 1 g sample protein in 1 minute at 37°C is defined as 1 unit.

PEPC activity (U/gprot) = $\Delta A340 \div (\varepsilon \times d) \times 10^6 \times V_1 \div V_2 \div C_{pr} \times f \div T$

Note:

ΔA340: Absolute OD value of the sample ($\Delta A340 = A_1 - A_2$).

ε: The molar extinction coefficient of NADH at 340nm, 6.22×10^3 L/mol/cm.

d: Optical path, 0.6 cm.

10^6: 1 mol = 10^6 μmol.

V_1: The volume of reaction system, 0.24 mL.

V_2: The volume of sample in reaction system, 0.04 mL.

C_{pr}: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

T: The time of reaction, 10 min.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three Shanghai green tissue samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	3.60	11.80	19.50
%CV	4.3	2.9	1.5

Inter-assay Precision

Three Shanghai green tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	3.60	11.80	19.50
%CV	10.6	8.5	9.4

Recovery

Three samples of high concentration, middle concentration and low concentration were tested with 6 parallel replicates for each concentration to obtain an average recovery rate of 103%.

Recovery

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	7.6	15.5	20
Observed Conc. (U/L)	8.4	14.9	20.4
Recovery rate (%)	111	96	102

Sensitivity

The analytical sensitivity of the assay is 0.72 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

12. Appendix II Example Analysis

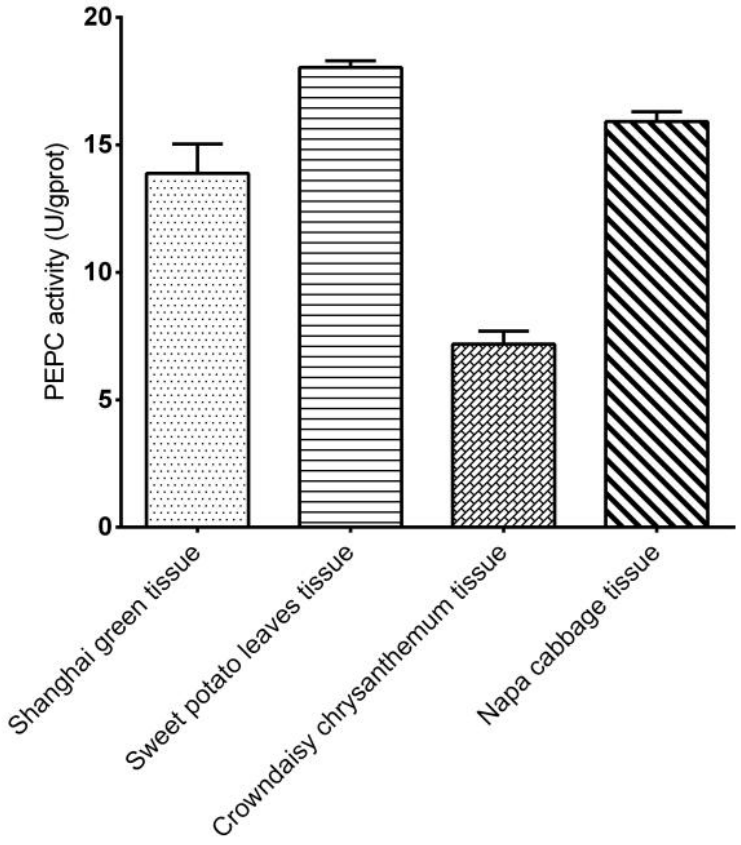
Example analysis:

For 10% Shanghai green tissue homogenate, take 40 µL and carry out the assay according to the operating steps. The results are as follows:

The average OD value of A_1 is 1.349, after incubation at 37°C for 10 min, the average OD value of A_2 is 1.244, ΔA340 = 1.349 - 1.244 = 0.105, the concentration of protein in sample is 3.03 gprot/L, and the calculation result is:

PEPC activity (U/gprot) = 0.105 ÷ (6.22 × 10^3 × 0.6) × 10^6 × 0.24 ÷ 0.04 ÷ 3.03 × 1 ÷ 10 = 55.71 U/gprot

Testing of 10% Shanghai green tissue homogenate (protein concentration: 3.03 gprot/L), 10% sweet potato leaves tissue homogenate (protein concentration: 1.75 gprot/L), 10% crowndaisy chrysanthemum tissue homogenate (protein concentration: 1.40 gprot/L) and 10% napa cabbage tissue homogenate (protein concentration: 0.92 gprot/L) according to the protocol yielded the following results:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.