（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K613-M

Specification：48T(48 samples)/96T(96 samples)

Measuring instrument：Microplate reader（320-360 nm）

Detection range：0.72-25.61 U/L

Elabscience®Phosphoenolpyruvate Carboxylase (PEPC) Activity Assay Kit

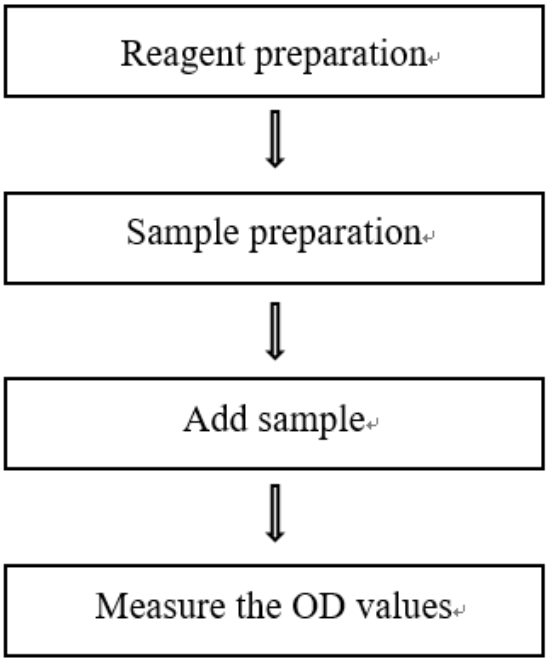
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary



Intended use

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

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 of absorbance value at 340 nm.

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℃, 12 months, shading light
Reagent 2	Substrate	Powder × 2 vials	Powder × 4 vials	2-8℃, 12 months, shading light
	UV Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (320-360 nm, optimum wavelength: 340 nm), Incubatro(37℃)

Reagents:

PBS (0.01 M，pH 7.4)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of working solution：

Dissolve one vial of substrate with 6 mL of buffer solution, mix well to dissolve.

Storage at 2-8 ℃ for 3 days protected from light.

Sample preparation

① Sample preparation

Tissue sample:

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℃.

④ Centrifuge at 10000×g for 10 minutes at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K168-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
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 do pretest to confirm the dilution factor.

The key points of the assay

It is recommended to use fresh plant samples for determination.

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.

③ Incubation at 37 ℃ for 10 min, measure the OD values of each well at 340 nm with microplate reader. Recorded as A_2. $\Delta A_{340} = A_1 - A_2$.

Calculation

The sample:

Tissue sample:

Unit definition: The enzyme amount of 1 μmol of NADH consumed by 1 g sample protein in 1 minute at 37 ℃ is defined as 1 unit.

$$\text{PEPC activity (U/gprot)} = \Delta A_{340} \div (\varepsilon \times d) \times 10\text{^}6 \times V_1 \div V_2 \div C_{pr} \times f \div T$$

[Note]

ΔA_{340}: Absolute OD value of the sample ($\Delta A_{340} = A_1 - A_2$).

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.

Appendix I Performance Characteristics

1. 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).

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.

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

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 103%.

	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.

Appendix II Example Analysis

Example analysis:

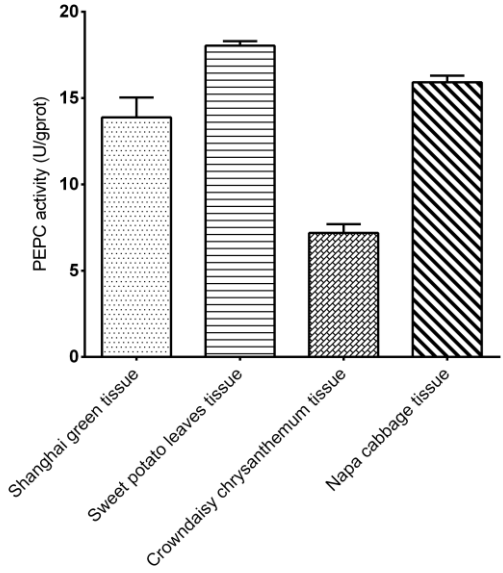
For 10% Shanghai green tissue homogenate, take 40 μL and carry the assay according to the operation steps. The results are as follows:

the average OD value of A_1 is 1.349, incubation at 37 ℃ for 10 min, the average OD value of A_2 is 1.244, ΔA_{340} = 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 ×10 ÷10

= 55.71 U/gprot

Detect 10% shanghai green tissue homogenate (the concentration of protein is 3.03 gprot/L), 10% sweet potato leaves tissue homogenate (the concentration of protein is 1.75 gprot/L), 10% crowndaisy chrysanthemum tissue homogenate (the concentration of protein is 1.40 gprot/L) and 10% napa cabbage tissue homogenate (the concentration of protein is 0.92 gprot/L) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.