



# **Phosphoenolpyruvate Carboxylase Activity Colorimetric Microplate Assay Kit User Manual**

**Catalog # CAK1076**

(Version 1.5E)

Detection and Quantification of Phosphoenolpyruvate  
Carboxylase(PEPC) Activityin Urine, Serum, Plasma, Tissue extracts,  
Cell lysate, Cell culture media and Other biological fluidsSamples.

**For research use only. Not for diagnostic or therapeutic procedures.**

|                                               |   |
|-----------------------------------------------|---|
| I. INTRODUCTION.....                          | 2 |
| II. KIT COMPONENTS.....                       | 3 |
| III. MATERIALS REQUIRED BUT NOT PROVIDED..... | 4 |
| IV. REAGENT PREPARATION.....                  | 5 |
| V. SAMPLE PREPARATION.....                    | 6 |
| VI. ASSAY PROCEDURE.....                      | 7 |
| VII. CALCULATION.....                         | 8 |
| VIII.TYPICAL DATA.....                        | 9 |

## I. INTRODUCTION

PEPC (phosphoenolpyruvate carboxylase), EC4.1.1.31, belongs to an enzyme family of carboxy-lyases that is catalyzing adding for carbon dioxide to phosphoenolpyruvate (PEP) to form oxaloacetate.

Phosphoenolpyruvate Carboxylase ActivityColorimetric Microplate Assay Kit provides a simple and direct procedure for measuring phosphoenolpyruvate carboxylase activity in a variety of samples. The formation of oxaloacetate is monitored spectrophotometrically in a malate dehydrogenase coupled system. The reaction velocity is measured as a decrease in 450nm resulting from the oxidation of NADH.

## II.KIT COMPONENTS

| Component         | Volume     | Storage |
|-------------------|------------|---------|
| 96-WellMicroplate | 1 plate    |         |
| Assay Buffer      | 30mlx 4    | 4 °C    |
| Diluent           | 20 mlx 1   | 4 °C    |
| Enzyme            | Powderx 1  | -20 °C  |
| Substrate         | Powder x 1 | -20 °C  |
| Dye ReagentA      | Powder x 1 | 4 °C    |
| Dye Reagent B     | 1 ml x 1   | 4 °C    |
| Standard          | Powder x 1 | -20 °C  |
| Positive Control  | Powder x 1 | -20 °C  |
| Technical Manual  | 1 Manual   |         |

### **III. MATERIALS REQUIRED BUT NOT PROVIDED**

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

#### IV. REAGENT PREPARATION

**Standard:** Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 2 mmol/L of standard stock solution, store at 4 °C for 1 day or -20°C for 3 days after reconstitution. Then dilute to 400  $\mu$ mol/L standard top solution by adding 0.2 ml stock solution into 0.8 ml distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 400/200/100/50/25/12.5/6.25  $\mu$ mol/L.

**Enzyme:** Briefly centrifuge prior to opening. Add 1 ml Diluent to dissolve before use. Store at -80°C for 1 month after reconstitution.

**Substrate:** Add 12 ml Diluent to dissolve before use. Keep in dark for immediate use or store at -20°C for 1 week after reconstitution.

**Positive Control:** Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use, then add 0.1 ml into 0.9 ml distilled water, mix. Store at -80°C for 1 month after reconstitution.

**Dye Reagent A:** Briefly centrifuge prior to opening. Add 5 ml distilled water to dissolve before use, mix, store at 4°C for 1 month after reconstitution.

**Note:** Divide into small aliquots to avoid repeated freeze-thaw cycles.

## V. SAMPLE PREPARATION

### 1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for  $5 \times 10^6$  cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

### 2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

### 3. For serum or plasma samples

Detect directly.

## VI. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents in the microplate:

| Reagent*                                                                                  | Sample**    | Control     | Positive Control | Standard    | Blank       |
|-------------------------------------------------------------------------------------------|-------------|-------------|------------------|-------------|-------------|
| Substrate                                                                                 | 120 $\mu$ l | 120 $\mu$ l | 120 $\mu$ l      | --          | --          |
| Enzyme                                                                                    | 10 $\mu$ l  | 10 $\mu$ l  | 10 $\mu$ l       | --          | --          |
| Sample                                                                                    | 10 $\mu$ l  | --          | --               | --          | --          |
| Distilled water                                                                           | --          | 10 $\mu$ l  | --               | 40 $\mu$ l  | 140 $\mu$ l |
| Positive Control                                                                          | --          | --          | 10 $\mu$ l       | --          | --          |
| Mix and incubate at room temperature for 2 minutes.                                       |             |             |                  |             |             |
| Standard                                                                                  | --          | --          | --               | 100 $\mu$ l | --          |
| Dye Reagent A                                                                             | 50 $\mu$ l  | 50 $\mu$ l  | 50 $\mu$ l       | 50 $\mu$ l  | 50 $\mu$ l  |
| Dye Reagent B                                                                             | 10 $\mu$ l  | 10 $\mu$ l  | 10 $\mu$ l       | 10 $\mu$ l  | 10 $\mu$ l  |
| Mix and incubate at room temperature for 2 minutes, record absorbance measured at 450 nm. |             |             |                  |             |             |

### Note:

\*Reagents must be added sequentially and should not be premixed prior to addition.

\*\*For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

\*\*For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.



## VII. CALCULATION

**Unit Definition:** One unit of PEPC activity is defined as the enzyme decomposes 1 μmol of NADH per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}) - \text{Intercept}}{\text{Slope} \times T} \times \frac{V_{\text{Standard}}}{V_{\text{Sample}}} \times n (\text{U/mL})$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times C_{\text{Protein}} \times T} (\text{U/mg})$$

2.2 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U}/10^4)$$

2.3 According to the weight of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U/g})$$

2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times T} (\text{U/mL})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C<sub>Protein</sub>: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10<sup>4</sup>

C<sub>Standard</sub>: the concentration of standard, μmol/L

V<sub>Standard</sub>: the volume of standard in assay procedure, mL

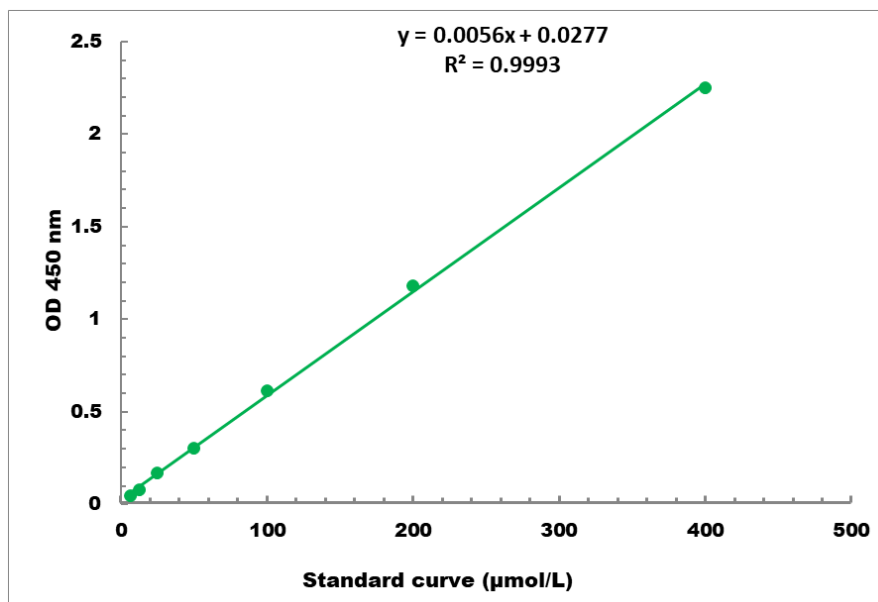
V<sub>Sample</sub>: the volume of sample in assay procedure, mL

$V_{\text{Assay}}$ : the volume of Assay Buffer in sample preparation, mL

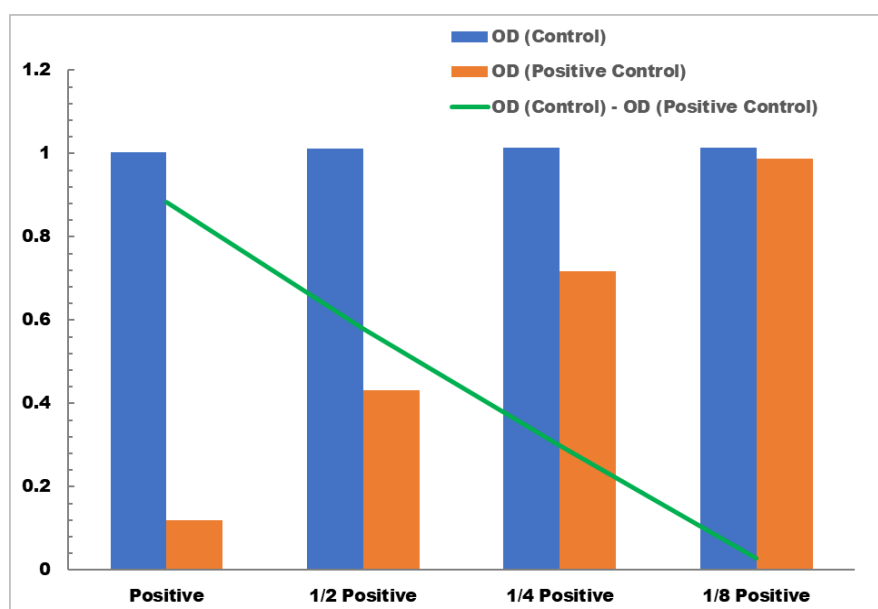
T: the reaction time, minute

## VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 4 μmol/L - 400 μmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration