



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

**Catalog # CAK1076**

(Version 1.3D)

Detection and Quantification of Phosphoenolpyruvate Carboxylase (PEPC) Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

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## I. INTRODUCTION

PEPC (phosphoenolpyruvate carboxylase), EC 4.1.1.31, belongs to an enzyme family of carboxy-lyases that is catalyzing adding for carbon dioxide to phosphoenolpyruvate (PEP) to form oxaloacetate.

The formation of oxaloacetate is monitored spectrophotometrically in a malate dehydrogenase coupled system. The reaction velocity is measured as a decrease in  $A_{340}$  resulting from the oxidation of NADH.

## II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Diluent	20 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Substrate	Powder x 1	-20 °C
Standard	Powder x 1	-20 °C
Positive Control	Powder x 1	-20 °C
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### Note:

**Enzyme:** add 1 ml Diluent to dissolve before use.

**Substrate:** add 18 ml Diluent to dissolve before use.

**Standard:** add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400  $\mu\text{mol/L}$ .

**Positive Control:** add 1 ml distilled water to dissolve before use, then add 0.1 ml into 0.9 ml distilled water, mix.

## III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 340 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

#### IV. 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.

## V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank	Positive Control
Standard	--	200 $\mu$ l	--	--
Distilled water	--	--	200 $\mu$ l	--
Substrate	180 $\mu$ l	--	--	180 $\mu$ l
Enzyme	10 $\mu$ l	--	--	10 $\mu$ l
Sample	10 $\mu$ l	--	--	--
Positive Control	--	--	--	10 $\mu$ l
Mix, measured at 340 nm and record the absorbance of 10th second and 130th second.				

### Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.
- 3) Reagents must be added step by step, can not be mixed and added together.

## VI. CALCULATION

**Unit Definition:** One unit of PEPC activity is defined as the enzyme decomposes 1  $\mu\text{mol}$  of NADH per minute.

1. According to the protein concentration of sample

$$\begin{aligned} \text{PEPC (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \\ &\quad / (V_{\text{Sample}} \times C_{\text{Protein}}) / T \\ &= 4 \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{PEPC (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times W / V_{\text{Assay}}) / T \\ &= 4 \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{PEPC (U}/10^4) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \\ &\quad / (V_{\text{Sample}} \times N / V_{\text{Assay}}) / T \\ &= 4 \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

4. According to the volume of serum or plasma

$$\begin{aligned} \text{PEPC (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \\ &\quad / V_{\text{Sample}} / T \\ &= 4 \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(130\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

$C_{\text{Standard}}$ : the standard concentration,  $400 \mu\text{mol/L} = 0.4 \mu\text{mol/ml}$ ;

$V_{\text{Standard}}$ : the volume of standard,  $200 \mu\text{l} = 0.2 \text{ ml}$ ;

$C_{\text{Protein}}$ : the protein concentration,  $\text{mg/ml}$ ;

$W$ : the weight of sample,  $\text{g}$ ;

$N$ : the quantity of cell or bacteria,  $N \times 10^4$ ;

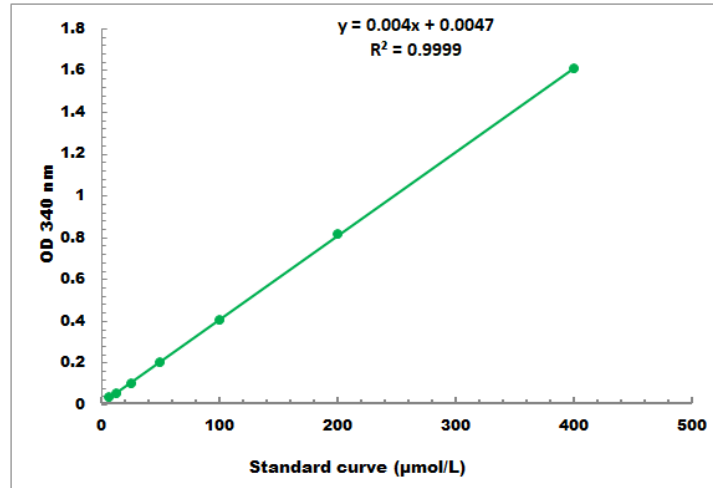
$V_{\text{Sample}}$ : the volume of sample,  $0.01 \text{ ml}$ ;

$V_{\text{Assay}}$ : the volume of Assay buffer,  $1 \text{ ml}$ ;

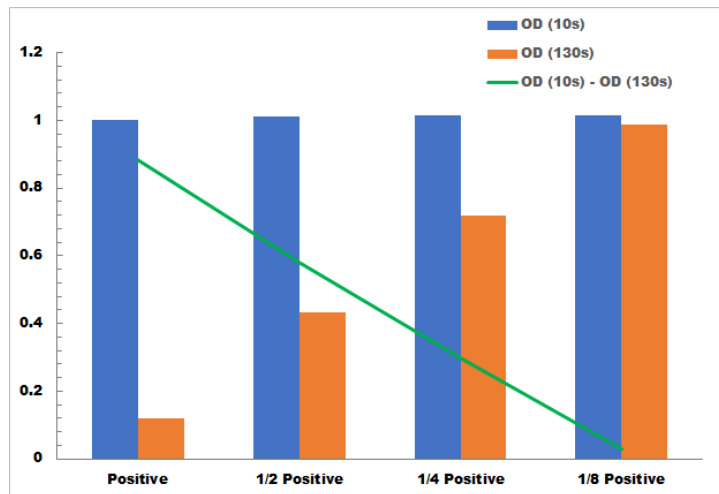
$T$ : the reaction time,  $2 \text{ minutes}$ .

## VII. 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

## VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to [www.cohesionbio.com](http://www.cohesionbio.com) or contact us at [techsupport@cohesionbio.com](mailto:techsupport@cohesionbio.com)

## IX. NOTES