Pyruvate Carboxylase

Microplate Assay Kit

Cat #: orb759229 (manual)

Detection and Quantification of Pyruvate Carboxylase (PC) 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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INTRODUCTION

Pyruvate carboxylase (EC 6.4.1.1) catalyzes the carboxylation of pyruvate to oxaloacetate, which is the precursor for the biosynthesis of many C4 intermediates and is used in gluconeogenesis, biosynthesis of amino acids, and fat metabolism. Pyruvate carboxylase belongs to the family of biotin-dependent carboxylases and is composed of four identical subunits (~ 130 kDa each) organized as a tetramer. It is present in many organisms including bacteria, fungi, plants, and animals. Pyruvate carboxylase is situated in mitochondria in most eukaryotic organisms. Biotin is an important regulator of pyruvate carboxylase activity. Long-term regulation of this enzyme in yeast can be realized by controlling the availability of biotin. Additionally, acetyl-CoA and l-aspartate could be used as the activator and inhibitor, respectively, for short-term regulation.

Pyruvate Carboxylase Microplate Assay Kit provides a simple and direct procedure for measuring pyruvate carboxylase levels 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 A340 resulting from the oxidation of NADH.

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
Technical Manual	1 Manual	

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 µmol/L.

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

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 5×10^6 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.

ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Standard	--	200 µl	--
Distilled water	--	--	200 µl
Sample	10 µl	--	--
Substrate	180 µl	--	--
Enzyme	10 µl	--	--
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) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

CALCULATION

Unit Definition: One unit of Pyruvate Carboxylase activity is defined as the enzyme decomposes 1 nmol of NADH per minute.

1. According to the protein concentration of sample

$$PC\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 4000 \times (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$PC\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 4000 \times (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$PC\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 4000 \times (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of serum or plasma

$$PC\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 4000 \times (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 400 μmol/L = 400 nmol/ml;

$V_{Standard}$: the volume of standard, 200 μl = 0.2 ml;

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

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

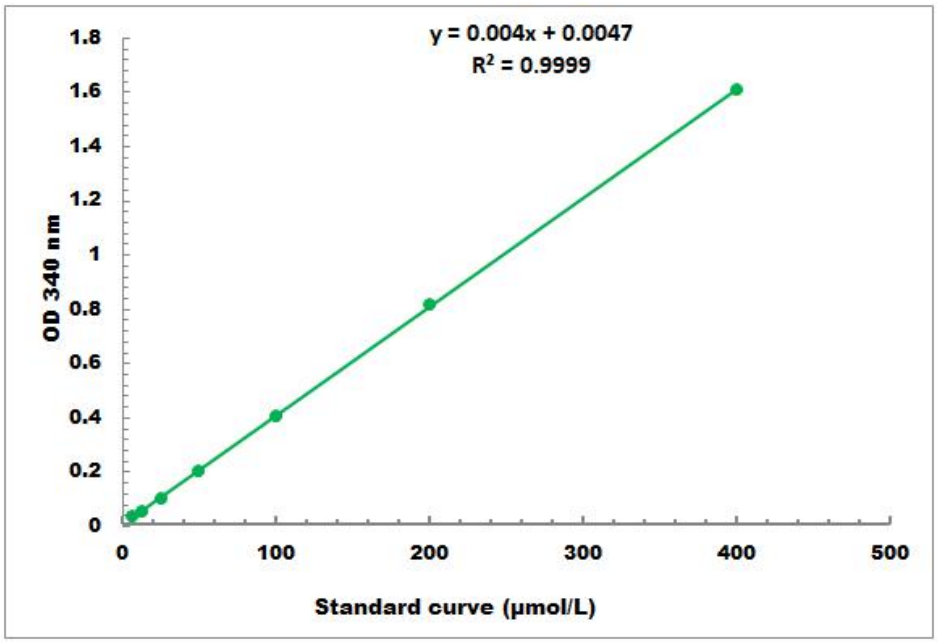
V_{Sample}: the volume of sample, 0.01 ml;

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

T: the reaction time, 2 minutes.

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

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