Pyruvate Carboxylase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1262

(Version 1.5B)

Detection and Quantification of Pyruvate Carboxylase(PC) 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

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 ActivityColorimetric 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 A450 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
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. REAGENTPREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 2 mmol/L of standardstock solution, store at 4 °C for 1 day or -20°C for 3daysafter reconstitution.Then dilute to400µmol/Lstandard top solutionby adding0.2 ml stock solution into 0.8 mldistilled water**.**Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be400/200/100/50/25/12.5/6.25µmol/L.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Diluent to dissolve before use.Keep in dark for immediate use or store at -20°C for 1 week after reconstitution.

Substrate: Add 12 ml Diluent to dissolve before use.Keep in dark for immediate use or store at -20 °C for 2-4 weeks.

Dye Reagent A: Briefly centrifuge prior to opening. Add 5ml 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 mlAssay 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

Weighout 0.1 g tissue, homogenize with 1 mlAssay 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 reagentsto room temperature before use.

Add following reagents in the microplate:

Reagent*	Sample**	Standard	Blank
Distilled water	--	40 µl	140 µl
Sample	10 µl	--	--
Substrate	120 µl	--	--
Enzyme	10 µl	--	--
Mix andincubate at room temperature for 5 minutes.			
Standard	--	100 µl	--
Dye Reagent A	50 µl	50 µl	50 µl
Dye Reagent B	10 µl	10 µl	10 µl
Mix andincubate 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 Pyruvate Carboxylaseactivity is defined as theenzymedecomposes 1μmol of NADH per minute.

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

2.4 According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

$C_{Standard}$: the concentration of standard, μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

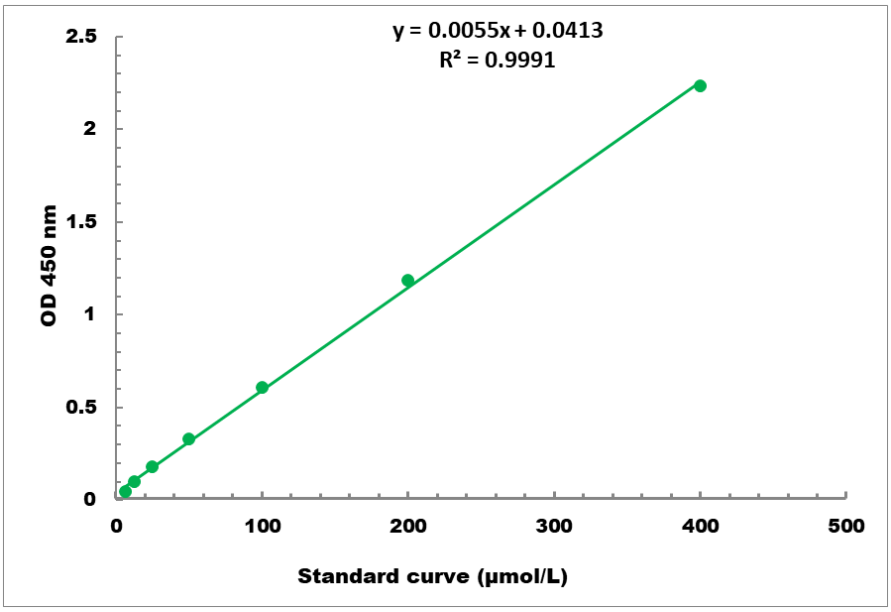
V_{Sample}: the volume of sample in assay procedure, mL

V_{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: 4μmol/L - 400 μmol/L