# Pyruvate CarboxylaseAssay Kit

ARG83429 Pyruvate Carboxylase Assay Kit can be used to measure Pyruvate Carboxylase in urine, serum, plasma, tissue extracts, cell lysate, cell culture media and other biological fluids.

Catalog number: ARG83429

Package: 96 wells

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## INTRODUCTION

Pyruvate carboxylase was first discovered in 1959 at Case Western Reserve University by M. F. Utter and D. B. Keech. Since then it has been found in a wide variety of prokaryotes and eukaryotes including fungi, bacteria, plants, and animals. In mammals, PC plays a crucial role in gluconeogenesis and lipogenesis, in the biosynthesis of neurotransmitters, and in glucose-induced insulin secretion by pancreatic islets. Oxaloacetate produced by PC is an important intermediate, which is used in these biosynthetic pathways. In mammals, PC is expressed in a tissue-specific manner, with its activity found to be highest in the liver and kidney (gluconeogenic tissues), in adipose tissue and lactating mammary gland (lipogenic tissues), and in pancreatic islets. Activity is moderate in brain, heart and adrenal gland, and least in white blood cells and skin fibroblasts.

## PRINCIPLE OF THE ASSAY

Pyruvate CarboxylaseAssay Kit determined Pyruvate Carboxylaseactivity in urine, serum, plasma, tissue extracts, cell lysate, cell culture media and other biological fluids. The assay kit base on the formation of oxaloacetate is monitored spectrophotometrically in a malate dehydrogenase coupled system. The reaction velocity oxidation of NADH can be measured at a colorimetric readout at 340 nm

## MATERIALS PROVIDED & STORAGE INFORMATION

| Component | Quantity | Storage |
|---|---|---|
| Microplate | 1 X 96-well plate | |
| Standard | 1 vial (lyophilized) | -20°C |
| Substrate | 1 vial (lyophilized) | -20°C |
| Enzyme | 1 vial (lyophilized) | -20°C |
| Assay Buffer | 4 X 30 ml | 4°C |
| Diluent | 20 ml | 4°C |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 340 nm
- Pipettes and pipette tips
- Deionized or distilled water

## TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store Assay Buffer and Diluent at 4°C, all other component at -20°C.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

- Avoid using reagents from different batches.
- Use only reagents from the same lot for each assay. This is especially important when running more than one plate per sample group.
- A separate standard curve must be run on each plate.

## SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

For cell and bacteria - Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5 × 106 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.

For tissue - 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.

Note: For other liquid sample, it can be assayed directly.

## REAGENT PREPARATION

- **Standard:** Reconstitute the Standard with **1 ml** of **distilled water** make sure the Standard is dissolved completely, then add **200 µl Standard buffer** into **800 µl distilled water.** The concentration will be 0.4 µmol/ml.

- **Substrate:** Reconstitute the Substrate with **18 ml** of **Diluent**. Allow the Substrate keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use.

- **Enzyme:** Reconstitute the Enzyme with **1 ml** of **Diluent**. Allow the Enzyme keep on bench for few minutes. Make sure the Enzyme is dissolved completely and mixed thoroughly before use.

## ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Add **10 µl Sample** into Sample wells.
2. Add **200 µl Standard** into Standard wells.
3. Add **180 µl Substrate** into Sample wells.
4. Add **10 µl Enzyme** into Sample wells.
5. Mix well, record the absorbance of 10$^{th}$ second and 130$^{th}$ at **OD 340 nm**.

Summary of Pyruvate CarboxylaseAssay Procedure

| Reagent | Sample | Standard | Blank |
|---|---|---|---|
| Standard | - | 200 µl | - |
| Distilled water | - | - | 200 µl |
| Sample | 10 µl | - | - |
| Substrate | 180 µl | - | - |
| Enzyme | 10 µl | - | - |
| Record the absorbance of 10$^{th}$ second and 130$^{th}$ at **OD 340 nm**. | | | |

## CALCULATION OF RESULTS

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

2. Calculate the average absorbance values for each set of samples and control.

3. Calculation:

A. Definition:

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

$C_{Standard}$: the concentration of Standard, 0.4 μmol/ml;

$V_{Sample}$: the volume of reaction sample, 10 μl = 0.01 ml;

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

$V_{assay}$: the volume of Assay Buffer, 1000 μl = 1 ml;

W: the weight of sample, g;

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

T: the reaction time, 2 minutes.

B. Formula:

a). According to the protein concentration of sample

Pyruvate Carboxylaseactivity (U/mg) =

$(OD_{Sample(10S)} - OD_{Sample(130S)}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times (C_{Protein} \times V_{Sample}) \times T]$

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

b). According to the weight of sample

Pyruvate Carboxylaseactivity (U/g) =

$(OD_{Sample(10S)} - OD_{Sample(130S)}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times (W \times V_{Sample}) / V_{Assay} \times T]$

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

c). According to the cell or bacteria

Pyruvate Carboxylaseactivity ($U/10^4$) =

$(OD_{Sample(10S)} - OD_{Sample(130S)}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times (N \times V_{Sample}) \times T]$

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

d). According to the volume of serum or plasma

Pyruvate Carboxylaseactivity (U/ml) =

$(OD_{Sample(10S)} - OD_{Sample(130S)}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T]$

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