# Pyruvate Carboxylase (PC) Assay Kit

**Catalog No.:** abx298982

**Size:** 96 tests

**Detection Range:** 6.43 U/L – 876.53 U/L

**Sensitivity:** 6.43 U/L

**Storage:** Store all components at -20°C. Store the Catalyst, Enzyme Reagent, Accelerating Reagent, and Reducing Reagent in the dark.

**Application:** For detection and quantification of Pyruvate Carboxylase activity in serum, plasma, animal tissue homogenates, and cell lysates.

## Introduction

Pyruvate Carboxylase is a biotin-containing enzyme that acts as a key mediator of eukaryotic metabolism, as part of the citric acid cycle. Pyruvate Carboxylase converts pyruvate to oxaloacetic acid via carboxylation, which is the main route by which oxaloacetate levels in the cycle are regulated. In addition, as Pyruvate Carboxylate has a central role in gluconeogenesis, and variety of other catabolic pathways. As such, Pyruvate Carboxylase is widely distributed in the cells of animals, and many fungi.

Abbexa's Pyruvate Carboxylase (PC) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Pyruvate Carboxylase activity. Pyruvate Carboxylase activity produces oxaloacetate, which reacts with the Reducing Reagent provided with the kit. The Reducing Reagent has an absorbance maximum at 340 nm. As such, the intensity of the absorbance is inversely proportional to the Pyruvate Carboxylase activity, which can then be calculated.

## Kit components

1. 96-well microplate
2. Assay Buffer: 30 ml
3. Catalyst: 2 vials
4. Enzyme Reagent: 0.2 ml
5. Accelerating Reagent: 0.2 ml
6. Reducing Reagent: 2 vials
7. Plate sealer: 2

## Materials required but not provided

1. Microplate reader (340 nm)
2. Double-distilled water
3. Normal saline (0.9% NaCl)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. 1.5 ml microcentrifuge tubes
7. Centrifuge
8. Vortex mixer
9. Incubator

## Protocol

### A. Preparation of samples and reagents

#### 1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum** and **Plasma:** Serum and plasma samples can be tested directly.
- **Animal Tissue Homogenates:** Carefully weigh out at least 20 mg of tissue, and wash in ice-cold PBS (0.01 M, pH 7.4). Add the tissue into normal saline (0.9% NaCl) in a ratio of 1 : 9 weight (mg) to volume (µl) (i.e. for 20 mg of tissue, add into 180 µl of normal saline (0.9% NaCl)). Homogenize manually, using a mechanical homogenizer or by ultrasonication, in an ice water bath. Take the resulting tissue homogenate, and centrifuge at 10,000 × g for 10 minutes at 4°C. Carefully take the supernatant, keep on ice, and assay immediately.

  **Note:** To calculate Pyruvate Carboxylase activity in tissue homogenates using the formula in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately.
- **Cell Lysates:** Collect at least $1\times10^6$ cells for analysis, and wash in PBS (0.01 M, pH 7.4). Add the cells into normal saline (0.9% NaCl) in a ratio of $1\times10^6$ : 200 cells to volume (µl) (i.e. for $1\times10^6$ cells, add into 200 µl of normal saline (0.9% NaCl)). Homogenize manually, using an ultrasonicator at 4°C (for example, in an ice water bath). Take the resulting cell lysate, and centrifuge at 10,000 × g for 10 minutes at 4°C to remove any insoluble material. Carefully take the supernatant, keep on ice, and assay immediately.

  **Note:** To calculate Pyruvate Carboxylase activity in cell lysates using the formula in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

| Sample Type | Dilution Factor |
|---|---|
| Rat and Mouse serum and plasma | 10 – 20 |
| 10% Mouse liver tissue homogenate | 90 – 110 |
| 10% Mouse heart tissue homogenate | 90 – 110 |
| 10% Mouse lung tissue homogenate | 70 – 80 |
| $1.92\times10^6$ Chinese hamster ovary (CHO) cells | 2 – 4 |
| $0.88\times10^6$ HeLa cells | 2 – 4 |

**Note:**

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

## 2. Reagents

- **Working Catalyst Solution:** Reconstitute one vial of Catalyst powder by adding 0.75 ml of double-distilled water. Mix well until the Catalyst has fully dissolved. The Working Catalyst Solution can be stored for up to 12 days at -20°C in the dark.
- **Working Reducing Reagent:** Reconstitute one vial of Reducing Reagent powder by adding 0.5 ml of double-distilled water. Mix well until the Reducing Reagent has fully dissolved. The Working Reducing Reagent can be stored for up to 7 days at -20°C.
- **Substrate Solution:** Prepare the Substrate Solution by mixing fully Assay Buffer, Working Catalyst Solution, Enzyme Reagent, Accelerating Reagent, and Working Reducing Reagent in a ratio of 181 : 10 : 1 : 1 : 7 volume. For example, to prepare 200 µl of Substrate Solution, mix fully 181 µl of Assay Buffer, 10 µl of Working Catalyst Solution, 1 µl of Enzyme Reagent, 1 µl of Accelerating Reagent, and 7 µl of Working Reducing Reagent.

  Prepare only as much Substrate Solution as required for the number of samples being tested. The Substrate Solution should be prepared just before use, and used within 24 hours. Keep on ice until just before use.

**Note:**

- Allow all reagents to equilibrate to room temperature before use.

## B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

1. Mark positions on the 96-well plate for each sample and blank. *It is strongly recommended to prepare all wells in duplicate.*
2. Add 10 µl of sample to each sample well.
3. Add 10 µl of normal saline (0.9% NaCl) to each blank well.
4. Prepare a timer, ensure the incubator has reached the correct temperature, and ensure the microplate reader is fully prepared to read the plate. Then, add 190 µl of Substrate Solution to all wells.
5. Tap the plate to mix fully, or shake with a microplate reader for 5 seconds. Immediately start a timer.
6. At 15 seconds, measure the OD of each well with a microplate reader at 340 nm. Record these values as "$OD_1$".
7. Immediately incubate the plate at 37°C for 5 minutes.
8. Measure the OD of each well with a microplate reader at 340 nm. Record these values as "$OD_2$".

**Note:**

- After adding the Substrate Solution, the reaction will proceed extremely quickly. The OD values of the wells must be read at 15 seconds – any delay may result in abnormally low OD value readings.
- The OD value of all samples in the second reading ("$OD_2$", after 5 minutes incubation at 37°C) must be > 0.2. If $OD_2$ values are ≤ 0.2, the samples should be diluted and re-tested.

### C. Calculation of Results

The activity of Pyruvate Carboxylase in each sample well can be derived with the following formulae:

#### 1. Serum and Plasma samples:

One unit of Pyruvate Carboxylase activity is defined as the amount of enzyme required for 1 L of serum or plasma to produce 1 µmol of product per minute at 25°C.

$$\text{Pyruvate Carboxylase activity (U/L)} = F \times \frac{OD_{340nm} \times V_{Reaction}}{M \times L_{Path} \times V_{Sample} \times T}$$

#### 2. Tissue Homogenates and Cell Lysates:

Pyruvate Carboxylase activity in tissue samples and cell lysates is calculated according to total protein concentration (which must be assayed separately).

One unit of Pyruvate Carboxylase activity is defined as the amount of enzyme required for 1 g of tissue or cell sample to produce 1 µmol of product per minute at 25°C.

$$\text{Pyruvate Carboxylase activity (U/g protein)} = F \times \frac{OD_{340nm} \times V_{Reaction}}{M \times L_{Path} \times V_{Sample} \times T \times C_{Protein}}$$

where:

| | |
|---|---|
| $OD_{340nm}$ | Difference in OD values of sample ($OD_1 - OD_2$) minus the difference in OD values of blank well ($OD_1 - OD_2$).<br>i.e. $[\text{Mean Sample } OD_1 - OD_2] - [\text{Mean Blank } OD_1 - OD_2]$ |
| M | Molar extinction coefficient of the enzyme product at 340 nm ($6.22 \times 10^3$ L/µmol/cm) |
| $L_{Path}$ | Optical path (0.5 cm) |
| $V_{Reaction}$ | Volume of the reaction system (0.2 ml) |
| $V_{Sample}$ | Volume of sample (0.01 ml) |
| $C_{Protein}$ | Concentration of protein in sample (g/L) |
| T | Time of the incubation (5 minutes) |
| F | The dilution factor of sample |



## Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.