Gentaur

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BioVision
BioVision Incorporated

Pyruvate Carboxylase Activity Assay Kit (Colorimetric)

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(Catalog # K2075-100; 100 assays; Store at -20 °C)

I. Introduction:

Pyruvate carboxylase (PC) catalyzes the irreversible carboxylation of pyruvate to oxaloacetate (OAA), depending on the species. It is a tetramer composed of four identical monomers each with a molecular weight of 125 kDa. OAA produced by PC is an important intermediate in numerous biological pathways. In mammals, PC plays an essential role in gluconeogenesis, lipogenesis, synthesis of neurotransmitters, and glucose-induced insulin secretion by pancreatic islets. During nutritional overload, PC levels are increased in the pancreatic β-cells to increase pyruvate cycling due to an increase in glucose levels. PC deficiency can lead to lactic acidosis due to the accumulation of lactic acid. **BioVision's Pyruvate Carboxylase Activity Assay Kit** provides a facile, rapid way to measure PC activity in various biological samples. In this kit, pyruvate is carboxylated to OAA, which is further detected by the oxidation of NADH. Therefore, a decrease in NADH absorbance at 340 nm is proportional to the amount of PC in samples. The assay is simple, sensitive, and can detect PC activity lower than 200 µU in samples.

Sodium Pyruvate + HCO_3^- + ATP —**PC** / **PC Cofactor**→ Oxalocetate —**PC Developer**→ **Decrease in Absorbance** (340 nm)

II. Application:

- Measurement of pyruvate carboxylase activity in various samples.

III. Sample Types:

- Cell Lysates: 3T3 cells, etc.
- Tissue Homogenates: Rat brain, etc.

IV. Kit Contents:

Components	K2075-100	Cap Code	Part Number
PC Assay Buffer	25 ml	WM	K2075-100-1
PC Substrate I	200 µl	Amber	K2075-100-2
PC Substrate II	1 vial	Orange	K2075-100-3
PC Cofactor	1 vial	Blue	K2075-100-4
PC Developer	1 vial	Green	K2075-100-5
NADH Standard	1 vial	Yellow	K2075-100-6
PC Positive Control	20 µl	Violet	K2075-100-7

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (plate reader)
- Dounce Tissue Homogenizer (BioVision Cat. # 1998)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20 °C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay. Use within two months after opening.

- **PC Assay Buffer:** Store at 4 °C or -20 °C. Bring to room temperature (RT) before use.
- **PC Substrate I:** Ready to use. Thaw at RT. Keep on ice while in use. Store at -20 °C.
- **PC Substrate II:** Reconstitute the vial in 220 µl dH_2O. Divide into aliquots and store at -20 °C. Keep on ice while in use. Avoid multiple free-thaw cycles.
- **PC Cofactor and PC Developer:** Reconstitute the vial in 220 µl PC Assay Buffer. Divide into aliquots and store at -20 °C. Keep on ice while in use. Avoid multiple free-thaw cycles.
- **NADH Standard:** Reconstitute the vial in 1 ml PC Assay Buffer to make 10 mM NADH Standard stock solution. Divide into aliquots and store at -20 °C. Keep on ice while in use. Avoid multiple free-thaw cycles.
- **PC Positive Control:** Ready to use. Thaw at RT. Divide into aliquots and store at -20 °C. Keep on ice while in use. Avoid multiple free-thaw cycles.

VII. PC Assay Protocol:

1. **Sample Preparation: For tissues or cells:** Homogenize tissues (10 mg) or pelleted cells (~5 x 10^5) with 100 µl ice-cold PC Assay Buffer using Dounce Tissue Homogenizer (BioVision Cat. # 1998) and keep on ice for 10-15 min. Centrifuge samples at 12,000 x *g* and 4 °C for 15 min and collect the supernatant. Dilute the supernatant 5-20 fold in PC Assay Buffer. Add 2-10 µl of the diluted samples into a 96-well clear plate designated as Sample and Sample Background Control.
   For Positive Control: Dilute PC Positive Control to 5 fold with PC Assay Buffer prior to the assay. Add 4-7 µl of diluted PC Positive Control into a parallel well(s) labeled as Positive Control. **For Reagent Background Control:** Add 50 µl PC Assay Buffer to a well labeled as Reagent Background Control.
   Adjust the volume of Sample(s), Sample Background Control, Positive Control wells to 50 µl/well with PC Assay Buffer.

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Notes:

a. For Unknown Samples, we recommend running several dilutions of the samples to ensure that the readings are within the Standard Curve range.

b. Do not re-use the diluted PC Positive Control.

2. **Standard Curve Preparation:** Add 0, 2, 4, 6, 8, 10 µl of 10 mM NADH Standard stock solution into a series of wells to generate 0, 20, 40, 60, 80, 100 nmole/well of NADH Standard respectively. Adjust the volume to 100 µl/well with PC Assay Buffer.

   Note: Standards can be prepared at the end of the incubation time.

3. **Substrate Hydrolysis:** Mix enough reagents for the number of assays to be performed. Prepare 50 µl of Reaction Mix as indicated in the table below:

	Reaction Mix	Background Mix
PC Assay Buffer	34 µl	36 µl
NADH Standard	8 µl	8 µl
PC Developer	2 µl	2 µl
PC Substrate II	2 µl	2 µl
PC Cofactor	2 µl	2 µl
PC Substrate I	2 µl	--

   Add 50 µl of Reaction Mix to each well containing Sample, Positive Control, and Reagent Background Control and 50 µl of Background Mix to Sample Background Control wells and mix well, protected from light. The total volume of each well including Sample, Sample Background Control, Positive Control, and Reagent Background Control is 100 µl.

   Note: Prepare Reaction Mix and Background Mix immediately before adding to the wells.

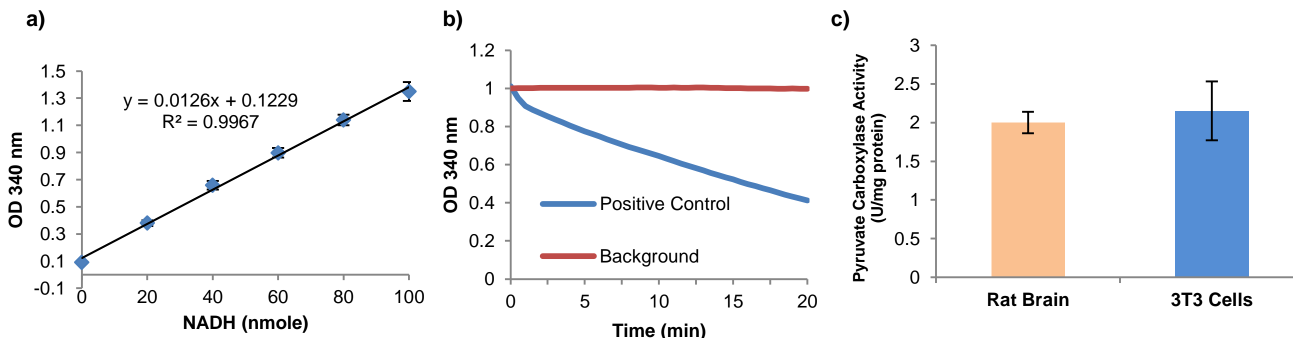
4. **Measurement:** Measure the absorbance of all wells at 37 °C for 20 min in kinetic mode at 340 nm. NADH Standard can be read in endpoint mode.

5. **Calculation:** Subtract 0 Standard reading from all Standard readings. Plot the NADH Standard Curve. Choose any two time points (t_1 & t_2) within the linear portion of the curve for each Sample type and obtain the corresponding absorbance values (OD_1 and OD_2; $OD_1 > OD_2$). Subtract OD_1 and OD_2 from the OD values of the corresponding Sample Background control well for the same time points. Apply the corrected Sample readings to the NADH Standard Curve to get B nmole of NADH consumed during the reaction time ($\Delta t = t_2 - t_1$).

Sample Pyruvate Carboxylase Activity = B x D / (Δt x V x M) = 1000 x nmole / min / mg = U / mg

Where: **B** = Amount of NADH consumed, calculated from the NADH Standard Curve (in nmole)
Δt = $t_2 - t_1$ (in min)
V = Sample volume added into the reaction well (µl)
M = Initial Sample concentration (mg protein / ml)
D = Sample dilution factor (D= 1 for undiluted samples)

Unit Definition: One unit of Pyruvate Carboxylase activity is the amount of enzyme that consumes 1.0 µmole of NADH per min, at pH 7.4 at 37 °C.



Figures: (a). NADH Standard Curve, results from multiple experiments. **(b).** Pyruvate Carboxylase activity of Positive Control. **(c).** PC activity in Rat Brain (2 µg protein) and 3T3 cells (2 µg protein) lysates. All assays were performed following kit protocol.

VIII. Related Products:

Pyruvate Carboxylase, Rhizobium etli Recombinant (P1661)
NADH Disodium salt trihydrate (2735)
Pyruvate Colorimetric/Fluorometric Assay Kit (K609)
Malate Dehydrogenase Activity Colorimetric Assay Kit (K654)
Dounce Tissue Homogenizer (1998)

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