

ab289840 – Pyruvate Carboxylase Activity Assay Kit (Colorimetric)

For the measurement of pyruvate carboxylase (PC) activity in cell lysates and tissue homogenates.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab289840>

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Storage and Stability

On receipt unopened assay kit can be stored at -20°C, protected from light. Once opened, use within 2 months.

Materials Supplied

Item	Quantity	Storage Condition
Pyruvate Carboxylase Assay Buffer/PC Assay Buffer	25 mL	-20°C
PC Substrate I	200 µL	-20°C
ATP II/PC Substrate II	1 vial	-20°C
Substrate IV/PC Cofactor	1 vial	-20°C
PC Developer Mix/PC Developer	1 vial	-20°C
NADH II/NADH Standard	1 vial	-20°C
PC Positive Control	20 µL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (plate reader)
- Dounce Tissue Homogenizer

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Pyruvate Carboxylase Assay Buffer/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.

ATP II/PC Substrate II: Reconstitute the vial in 220 µL dH₂O. Divide into aliquots and store at -20°C. Keep on ice while in use. Avoid multiple free-thaw cycles.

Substrate IV/PC Cofactor and PC Developer Mix/PC Developer: Reconstitute the vial in 220 µL Pyruvate Carboxylase Assay Buffer/PC Assay Buffer. Divide into aliquots and store at -20°C. Keep on ice while in use. Avoid multiple free-thaw cycles.

NADH II/NADH Standard: Reconstitute the vial in 1 mL Pyruvate Carboxylase Assay Buffer/PC Assay Buffer to make 10 mM NADH II/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.

Sample Preparation

For tissues or cells:

Homogenize tissues (10 mg) or pelleted cells (~5 x 10⁵) with 100 µL ice-cold Pyruvate Carboxylase Assay Buffer/PC Assay Buffer using Dounce Tissue Homogenizer 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 Pyruvate Carboxylase Assay Buffer/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 the Positive Control to 5-fold using Pyruvate Carboxylase Assay Buffer/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 Pyruvate Carboxylase Assay Buffer/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 Pyruvate Carboxylase Assay Buffer/PC Assay Buffer.

ΔNote: For Unknown Samples, we recommend running several dilutions of the samples to ensure that the readings are within the Standard Curve range. Do not re-use the diluted PC Positive Control.

Standard Curve Preparation

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

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

Substrate Hydrolysis

1. 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
Pyruvate Carboxylase Assay Buffer/PC Assay Buffer	34 µL	36 µL
NADH II/NADH Standard	8 µL	8 µL
PC Developer Mix/PC Developer	2 µL	2 µL
ATP II/PC Substrate II	2 µL	2 µL
Substrate IV/PC Cofactor	2 µL	2 µL
PC Substrate I	2 µL	-

2. 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.
3. The total volume of each well including Sample, Sample Background Control, Positive Control, and Reagent Background Control is 100 µL

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

Measurement

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

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 = $B \times D / (\Delta t \times V \times M) = 1000 \times \text{nmole} / \text{min} / \text{mg} = U / \text{mg}$

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.

Technical Support

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