



Phosphoenolpyruvate Carboxykinase Activity Assay Kit (Colorimetric)

rev. 1/21

(Catalog # K359-100; 100 assays; Store at -20 °C)

I. Introduction:

Phosphoenolpyruvate Carboxykinase (PEPCK EC 4.1.1.32) is an enzyme, which belongs to the lyase family. In the presence of GTP, it catalyzes the reversible conversion of oxaloacetate (OAA) into phosphoenolpyruvate (PEP), GDP and CO₂. In humans, two isoforms of PEPCK are found: cytosolic form (PEPCK-C, also called PCK1) and mitochondrial form (PEPCK-M). PEPCK-C is a rate-controlling step in gluconeogenesis. Recent studies found abnormal concentrations of PEPCK in diabetic mice. Therefore, accurate measurement of Phosphoenolpyruvate Carboxykinase activity is valuable for both, mechanistic and therapeutic studies. **BioVision's Phosphoenolpyruvate Carboxykinase (PEPCK) Activity Assay Kit** provides a quick and easy way for measuring PEPCK activity in various samples. In this assay, Phosphoenolpyruvate Carboxykinase is coupled with a set of enzymes that convert PEP and carbonate into a series of intermediates and hydrogen peroxide, which in turn, reacts with a probe and converted generating a colorimetric signal (OD: 570 nm). The color intensity is directly proportional to the amount of active Phosphoenolpyruvate Carboxykinase present in samples. The assay is simple, sensitive, high-throughput adaptable and can detect less than 10 µU of Phosphoenolpyruvate Carboxykinase activity per sample.

	Phosphoenolpyruvate Carboxykinase	Converter	Developer + Probe	
PEP + GDP + CO ₂	● OAA + GTP	▶	Pyruvate — Color detection (OD: 570 nr	n)

II. Applications:

- Measurement of Phosphoenolpyruvate Carboxykinase activity in various samples.
- Analysis of gluconeogenesis pathway.

III. Sample Types:

- Tissue samples: Rat liver, kidney, heart etc.
- Adherent/Suspension Cells: HeLa, Jurkat, HEK 293 cells etc.

IV. Kit Contents:

Components	K359-100	Cap Code	Part Number
PEPCK Assay Buffer	25 ml	WM	K359-100-1
PEPCK Probe (in DMSO)	0.2 ml	Red	K359-100-2
PEPCK Substrate Mix	1 vial	Orange	K359-100-3
PEPCK Converter	1 vial	Purple	K359-100-4
PEPCK Developer	1 vial	Green	K359-100-5
PEPCK Positive Control	1 vial	Blue	K359-100-6
Pyruvate Standard (100 nmol/µl)	100 µl	Yellow	K359-100-7

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- Dounce Homogenizer
- 30% Glycerol

VI. Storage, Handling and Reagent Preparation:

- PEPCK Assay Buffer: Warm PEPCK Assay Buffer to room temperature (RT) before use. Store at 4 °C.
- PEPCK Substrate, PEPCK Converter and PEPCK Developer: Reconstitute each vial with 220 µl PEPCK Assay Buffer. Pipette up and down to dissolve completely. Store at -20 °C. Keep on ice while in use. Use within two months.
- PEPCK Probe (in DMSO): Ready to use as supplied. Thaw the DMSO at RT and mix well. Store at -20 °C. Use within two months.
- PEPCK Positive Control: Reconstitute in 40 µl of 30% Glycerol. Store at -20 °C. Keep on ice while in use. Use within two months.
- Pyruvate Standard: Store at -20 °C. Keep on ice while in use. Use within two months.

VII. PEPCK Activity Assay Protocol:

1. Sample Preparation: Homogenize tissue (20 mg) or cells (2 x 10⁶) with 200 µl ice cold PEPCK Assay Buffer for 10 min on ice. Centrifuge at 10,000 x g at 4° C for 10 min. Collect the supernatant and measure the protein concentration. Add 2-50 µl sample per well. Adjust the final volume to 50 µl with PEPCK Assay Buffer. For Positive Control, add 2-10 µl of reconstituted PEPCK Positive Control, and adjust the final volume to 50 µl with PEPCK Assay Buffer.

Notes:

- a. For Unknown Samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.
- b. Sample Background Control (with sample but without the PEPCK Substrate Mix) allows for the correction of non-specific sample background. Adjust the volume to 50 µl with PEPCK Assay Buffer.
- 2. Pyruvate Standard Curve: Dilute Pyruvate Standard to 1 mM by taking 10 μl of 100 mM Pyruvate into 990 μl of PEPCK Assay Buffer, mix well. Add 0, 2, 4, 6, 8 and 10 μl of the 1 nmol/μL Pyruvate Standard into a series of wells in a 96 well clear microplate to generate 0, 2, 4, 6, 8 and 10 nmol/well. Adjust the volume to 50 μl/well with PEPCK Assay Buffer and mix well.





3. Reaction Mix: Make enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Standard Mix	Reaction Mix	Sample Control Mix*
PEPCK Assay Buffer	46 µl	42 µl	44 µl
PEPCK Converter		2 µl	2 µl
PEPCK Developer	2 µl	2 µl	2 µl
PEPCK Probe	2 µl	2 µl	2 µl
PEPCK Substrate Mix		2 µl	

Mix well. Add 50 µl of the Standard Mix to Pyruvate Standard wells and add 50 µl of the Reaction Mix to each well containing test samples. *For samples having high background, add 50 µl of Sample Control Mix to Sample Background Control well(s). Mix well.

4. Measurement: Measure the plate at OD 570 nm in kinetic mode at 37 °C for 10-60 min.

Note: We recommend measuring the fluorescence in kinetic mode, and choosing two time points $(t_1 \& t_2)$ in the linear range to calculate PEPCK Activity in samples. The Pyruvate Standard Curve can be read in Endpoint mode (i.e., at the end of incubation time).

5. Calculation: Subtract the 0 Standard reading from all readings. Plot the Pyruvate Standard Curve. If the Sample Background is high, subtract the Background Control reading from Sample reading. Calculate the PEPCK activity of the Test Sample. Determine the Δ OD (Δ OD = OD₂- OD₁) at linear range of two time point ($t_1 \& t_2$) and apply the Δ OD to the Pyruvate Standard Curve to get B nmol of Pyruvate generated by PEPCK at the reaction time (Δ t = t_2 - t_1).

Sample PEPCK Activity = B/(Δ T X V) x D = nmol/min/µl = mU/µl

Where: $\mathbf{B} = Pyruvate$ amount from the Standard Curve (pmol).

- **ΔT** = Time (min).
 - V = Sample volume added into the reaction well (µl).
 - **D** = Sample dilution factor

Unit Definition: One unit of Phosphoenolpyruvate Carboxykinase is the amount of enzyme that will generate 1.0 µmol of pyruvate per min at pH 7.5 at 37 °C.



Figure: A. Pyruvate Standard Curve. **B.** Phosphoenolpyruvate Carboxykinase (PEPCK) kinetic activity measured in Lysates: HEK 293 cells (66 μg), Rat Liver (223 μg) and Rat Kidney (145 μg). **C.** PEPCK Specific Activity in biological samples. Assays were performed following kit protocol.

VIII. RELATED PRODUCTS:

Glucose and Sucrose Assay (K616) Glucose Uptake Colorimetric Assay (K676) Glucose Uptake Fluorometric Assay (K666) Maltose and Glucose Assay (K618) Glucose-6-Phosphate dehydrogenase Activity Assay (K757) Glucose Dehydrogenase Activity Assay (K786) Oxalate Decarboxylase Colorimetric Assay (K664) Glucose-6-Phosphate Assay (K657) Glucose Assay kit II (K686) Oxalate (Oxalic Acid) Colorimetric Assay (K663) Ascorbic Acid Colorimetric Assay II (K671) Ascorbic Acid Colorimetric Assay (K661)

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