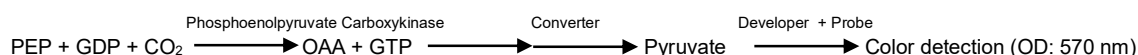


Phosphoenolpyruvate Carboxykinase Activity Assay Kit (Colorimetric)

(Catalog # BN00622; 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. Assay Genie's Phosphoenolpyruvate Carboxykinase (PEPCK) Activity Assay kit provides a quick and easy way for measuring PEPCK activity in various samples. In this assay, Phosphoenolpyruvate Carboxylase 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 Carboxylase present in samples. The assay is simple, sensitive, high-throughput adaptable and can detect less than 10 µU of Phosphoenolpyruvate Carboxykinase activity per sample.



II. Application:

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

III. Sample Type:

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

IV. Kit Contents:

Components	BN00622	Cap Code	Part Number
PEPCK Assay Buffer	25 ml	WM	BN00622-1
PEPCK Probe (in DMSO)	0.2 ml	Red	BN00622-2
PEPCK Substrate Mix	1 vial	Orange	BN00622-3
PEPCK Converter	1 vial	Purple	BN00622-4
PEPCK Developer	1 vial	Green	BN00622-5
PEPCK Positive Control	1 vial	Blue	BN00622-6
Pyruvate Standard (100 nmol/µl)	100 µl	Yellow	BN00622-7

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 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:** Ready to use as supplied. Thaw the probe solution in DMSO at room temperature and mix well, Store at -20°C. Use within two months.
- **PEPCK Positive Control:** Reconstitute with 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 Assay Buffer for 10 minutes 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 final volume to 50 µl with Assay Buffer. For positive control, add 2-10 µl of reconstituted PEPCK positive control, and adjust final volume to 50 µl with Assay Buffer.

Note:

- a. For unknown samples, we suggest testing several doses to ensure the readings are within the standard curve range.
- b. Sample background controls (with sample but without the PEPCK Substrate Mix) allow for 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 96 well clear microplate to generate 0, 2, 4, 6, 8 and 10 nmol/well. Adjust volume to 50 µl/well with PEPCK Assay Buffer, 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*
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PEPCK Assay Buffer	46 μ l	42 μ l	44 μ l
PEPCK Converter	--	2 μ l	2 μ l
PEPCK Developer	2 μ l	2 μ l	2 μ l
PEPCK Probe	2 μ l	2 μ l	2 μ l
PEPCK Substrate Mix	--	2 μ l	--

Mix well. Add 50 μ l of the Standard Mix to Pyruvate Standard Curve, Add 50 μ l of the Reaction Mix to each well containing test samples.

*For samples having high background, add 50 μ l of Sample Control Mix to sample background control well(s). Mix well.

4. Measurement: Measure the plate at OD 570nm 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 ΔOD ($\Delta OD = OD_2 - OD_1$) at linear range of two time point (t_1 & t_2), apply the ΔOD to the Pyruvate standard curve to get B nmol of Pyruvate generated by PEPCK at the reaction time ($\Delta t = t_2 - t_1$).

$$\text{Sample PEPCK Activity} = B / (T \times V) \times D = \text{nmol/min}/\mu\text{l} = \text{mU}/\mu\text{l}$$

Where: **B** = Pyruvate amount from the standard curve (pmol).

Δt = time (min).

V = sample volume added into the reaction well (μ l).

D = Sample dilution factor

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

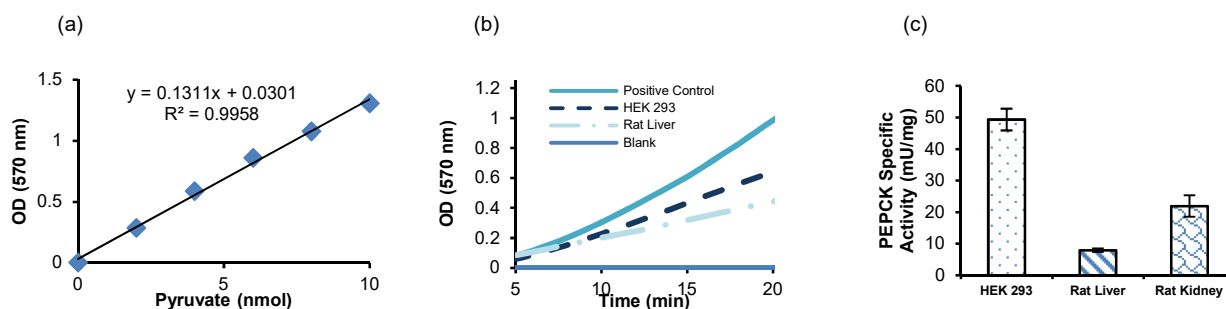


Figure: **A.** Pyruvate Standard Curve. **B.** Phosphoenolpyruvate Carboxykinase (PEPCK) kinetic activity measured in Lysates: HEK 293 cells (66 μ g), Rat Liver (223 μ g) and Rat Kidney (145 μ g). **C.** PEPCK Specific Activity in biological samples. Assays were performed following kit protocol.

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