



Phosphoenolpyruvate Carboxykinase 1 Inhibitor Screening Kit

(Catalog # K2012-100; 100 assays; Store at -20°C)

8/19

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, PEPCK1 or PCK1) and mitochondrial form (PEPCK-M). PEPCK1 is a rate-controlling step in gluconeogenesis and a key gluconeogenic enzyme in liver and kidney. Recent studies have shown that overexpressing of PEPCK1 in liver leads to excessive glucose production and type II diabetic phenotype in mice. Therefore, identifying and screening PEPCK1 inhibitors is a useful strategy for the treatment of type II diabetes and obesity. In BioVision's Human Phosphoenolpyruvate Carboxykinase 1 Inhibitor Screening Kit, PEPCK1 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 thereby generating a colorimetric signal (OD 570 nm). In the presence of PEPCK1 Inhibitor, the reaction is impeded. A PEPCK1 Inhibitor Control is included to compare the efficacy of the sample inhibitors. The assay is high-throughput adaptable and can be completed in less than 1 hr.



II. Application:

- Screening/characterizing/studying potential inhibitors of Human PEPCK1

III. Kit Contents:

Components	K2012-100	Cap Code	Part Number
PEPCK Assay Buffer	25 ml	WM	K2012-100-1
PEPCK Probe	200 µl	Red	K2012-100-2
PEPCK Substrate Mix	1 vial	Orange	K2012-100-3
PEPCK Converter	1 vial	Purple	K2012-100-4
PEPCK Developer	1 vial	Green	K2012-100-5
Human PEPCK1	1 vial	Blue	K2012-100-6
PEPCK Inhibitor Control	1 vial	Brown	K2012-100-7

IV. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom.
- Multi-well spectrophotometer (plate reader)
- Multi-channel pipette
- 30% Glycerol in dH₂O

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. After thawing, briefly centrifuge all small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

- **PEPCK Assay Buffer:** Warm PEPCK Assay Buffer to room temperature (RT) before use. Store at 4°C.
- **PEPCK Substrate Mix, 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. Use within two months.
- **PEPCK Probe (in DMSO):** Ready to use as supplied. Thaw the solution at RT. Mix well. Store at -20 °C. Use within two months.
- **Human PEPCK1:** Reconstitute the vial in 220 µl of 30% Glycerol. Mix well and set on ice for 10 min to completely dissolve. Avoid repeated freeze/thaw cycles. Aliquot and store at -80°C. Use within two months. Keep on ice while in use.
- **PEPCK Inhibitor Control:** Reconstitute the vial in 440 µl dH₂O and mix well. Aliquot and store at -20 °C. Use within two months.

VI. PEPCK1 Inhibitor Screening Protocol:

- Candidate Inhibitor Preparation:** Dissolve candidate Inhibitor(s) in appropriate solvent at highest concentration to be tested. Dilute to 2X the desired test concentration with PEPCK Assay Buffer. Add 50 µl diluted Candidate Inhibitor(s) into desired wells of a 96-well clear plate designated as Sample (S).
- Enzyme Control, Background Control, Solvent Control and Inhibitor Control Preparation:** For Enzyme Control (EC) and Background Control (BC), add 50 µl PEPCK Assay Buffer in each well. For PEPCK Inhibitor Control (IC), add 50 µl of PEPCK Inhibitor Control into desired well(s).
Note: If solvent is required to solubilize the candidate inhibitor(s), the final in-well solvent concentration might affect the enzymatic activity. Prepare a Solvent Control (SC) well with 50 µl of the final Solvent concentration in PEPCK Assay Buffer.
- Human PEPCK1 Enzyme:** Make enough PEPCK1 Enzyme for the number of assays to be performed. Add 2 µl of the reconstituted PEPCK1 Enzyme into S, EC, SC and IC wells. Add 2 µl PEPCK Assay Buffer into BC wells. Incubate at 25°C for 5 min.



4. Substrate Mix Preparation: Make enough reagents for the number of assays to be performed. For each well, prepare 50 μ l of Substrate Mix containing:

<u>Substrate Mix</u>	
PEPCK Assay Buffer	42 μ l
PEPCK Substrate	2 μ l
PEPCK Converter	2 μ l
PEPCK Developer	2 μ l
PEPCK Probe	2 μ l

Mix and add 50 μ l of Substrate Mix into EC, SC, IC, BC & S wells respectively. Mix well with gentle shaking.

5. Measurement: Measure OD 570 nm in a kinetic mode for 15-60 min at 37°C.

6. Calculations: Choose any two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values for the OD 570 nm (OD_1 & OD_2). Calculate the slope for Samples, Enzyme Control as 100%, by dividing the net ΔOD 570 ($OD_2 - OD_1$) value by the time Δt ($t_2 - t_1$). If Solvent Control (SC) value is significantly different than that of EC replace the value of EC in the formulas below with the SC values. Calculate % relative inhibition as follows:

$$\text{Relative Activity (\%)} = [(\text{Slope of S})/(\text{Slope of EC})] \times 100$$

$$\text{Relative Inhibition (\%)} = [(\text{Slope of EC} - \text{Slope of S})/(\text{Slope of EC})] \times 100$$

Where: **Slope of EC is the slope of Enzyme Control**

Slope of S is the slope of Candidate Inhibitor

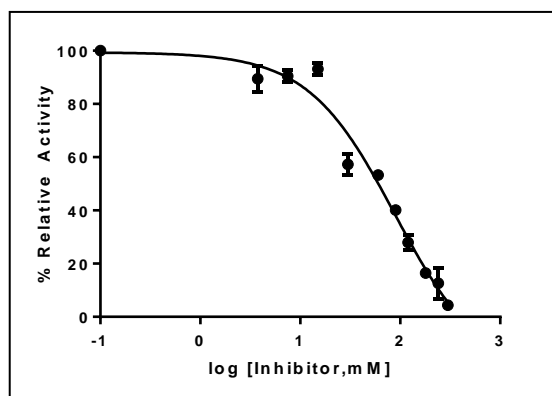


Figure: Inhibition of Human PEPCK1 activity by PEPCK1 Inhibitor ($IC_{50} = 90$ mM). Assay was performed following the kit protocol.

VII. Related Products:

- Phosphoenolpyruvate Carboxykinase Activity Assay Kit (Colorimetric) (K359)
- PEP Colorimetric/Fluorometric Assay Kit (K365)
- Phosphoenolpyruvate Carboxylase Activity Assay Kit (Colorimetric) (K443)
- PPDK (Pyruvate, phosphate dikinase) Activity Assay Kit (Fluorometric/Colorimetric) (K456)
- Pyruvate Kinase Activity Colorimetric/Fluorometric Assay Kit (K709)
- 2-Phosphoglycerate Colorimetric/Fluorometric Assay Kit (K778)

FOR RESEARCH USE ONLY! Not to be used on humans.