Technical Bulletin

Phosphoenolpyruvate Carboxykinase Activity Assay Kit (Colorimetric)

Catalog Number MAK408

Product Description

Phosphoenolpyruvate Carboxykinase (PEPCK) 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 CO_2. In humans, two isoforms of PEPCK are found: cytosolic form (PEPCK-C, also called PCK1) and mitochondrial form (PEPCK-M). PEPCK-C catalysis 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.

The Phosphoenolpyruvate Carboxykinase Activity Assay Kit provides a quick and easy way for measuring PEPCK activity in various samples. In this assay, PEPCK is coupled with a set of enzymes that convert phosphoenolpyruvate and carbonate into a series of intermediates and hydrogen peroxide, which in turn, reacts with a probe and generates a colorimetric signal measurable at 570 nm. The color intensity is directly proportional to the amount of active Phosphoenolpyruvate Carboxykinase present. The assay is simple, sensitive, and suitable for high-throughput applications. The method can detect less than 10 μU of PEPCK activity per sample.

The kit is suitable for the measurement of Phosphoenolpyruvate Carboxykinase activity in various samples including tissue (rat liver, kidney, heart, etc.) and adherent/suspension cells (HeLa, Jurkat, HEK 293 cells, etc.), as well as used for the analysis of the gluconeogenesis pathway.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- PEPCK Assay Buffer 25 mL
  Catalog Number MAK408A
- PEPCK Probe (in DMSO) 200 µL
  Catalog Number MAK408B
- PEPCK Substrate Mix 1 vial
  Catalog Number MAK408C
- PEPCK Converter 1 vial
  Catalog Number MAK408D
- PEPCK Developer 1 vial
  Catalog Number MAK408E
- PEPCK Positive Control 1 vial
  Catalog Number MAK408F
- Pyruvate Standard (100 nmol/µL) 100 µL
  Catalog Number MAK408G

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
- 96-well clear flat-bottom plate. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric multiwell plate reader
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Glycerol (Catalog Number G7757 or equivalent)
- Bicinchoninic Acid Kit for Protein Determination (Catalog Number BCA1 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store kit at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials at low speed prior to opening.

30% Glycerol: Prepare 30% glycerol by adding 3 parts of glycerol (not included) with 7 parts of purified water. Mix well.

PEPCK Assay Buffer: Ready to use as supplied. Warm to room temperature prior to use. Chill an appropriate amount of PEPCK Assay Buffer for use in Sample Preparation. Store at 2-8 °C.

PEPCK Substrate, PEPCK Converter and PEPCK Developer: Reconstitute each vial with 220 µL of PEPCK Assay Buffer. Pipette up and down to dissolve completely. Store at -20 °C. Keep on ice while in use. Use within two months once reconstituted.

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: Ready to use as supplied. Store at -20 °C. Keep on ice while in use. Use within two months

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

1. Homogenize tissue (20 mg) or cells (2×10^6 cells) with 200 µL of ice-cold PEPCK Assay Buffer for 10 minutes on ice.
2. Centrifuge at 10,000 × *g* at 4 °C for 10 minutes.
3. Collect the supernatant and measure the protein concentration.
4. Add 2-50 µL of Sample (S) to the plate well(s). Adjust the total volume to 50 µL with PEPCK Assay Buffer. For unknown samples, test different amounts of Sample to ensure the readings are within the Standard Curve Range.

Positive Control

To desired well(s), add 2-10 µL of reconstituted PEPCK Positive Control. Adjust total volume to 50 µL with PEPCK Assay Buffer.

Sample Background Control (SBC)

Running a Sample Background Control allows for the correction of non-specific sample background. Use the same amount of tissue/cell homogenate for each Sample Background Control (SBC) as for the paired Sample (S) well. Adjust the total volume to 50 µL per well with PEPCK Assay Buffer.

Standard Curve Preparation

Prepare a 1 mM Pyruvate Standard solution by diluting 10 µL of Pyruvate Standard (100 nmol/µL; 100 mM) with 990 µL of PEPCK Assay Buffer and mix well. Prepare Pyruvate Standards according to Table 1.

Table 1.
Preparation of Pyruvate Standards

Well	1 mM Pyruvate Standard	PEPCK Assay Buffer	Pyruvate (nmol/well)
1	0 µL	50 µL	0
2	2 µL	48 µL	2
3	4 µL	46 µL	4
4	6 µL	44 µL	6
5	8 µL	42 µL	8
6	10 µL	40 µL	10

Reaction Mixes

1. Mix enough reagents for the number of assays to be performed.
   a. For each well containing Standard, prepare 50 µL of Standard Mix according to Table 2. Mix well.
   b. For each well containing Sample (S) and Positive Control, prepare 50 µL of Reaction Mix according to Table 2. Mix well.
   c. For each well containing Sample Background Control (SBC), prepare 50 µL of Sample Background Control Mix according to Table 2. Mix well.

Table 2.
Preparation of Reaction Mixes

Reagent	Standard Mix	Reaction Mix	Sample Background 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	-

2. Add 50 µL of the Standard Mix to all Pyruvate Standard Curve wells. Mix well.
3. Add 50 µL of the Reaction Mix to each Sample (S) and Positive Control wells. Mix well.
4. Add 50 µL of the Sample Background Control Mix to each Sample Background Control (SBC) well. Mix well.

Measurement

Measure the plate at 570 nm (A_{570}) in kinetic mode at 37 °C for 10-60 minutes. The Pyruvate standard curve can be read in endpoint mode at the end of the incubation time.

Results

1. Subtract the 0 Standard A_{570} reading from all A_{570} readings, including Sample(s) (S), Sample Background Control (SBC), and Positive Controls.
2. Plot the Pyruvate standard curve.
3. Choose two time points (T_1 and T_2) in the linear range to calculate PEPCK Activity in Sample(s) (S).
4. If the Sample Background Control (SBC) A_{570} readings are high, subtract the Sample Background Control (SBC) A_{570} reading from its paired Sample (S) A_{570} reading.
5. Calculate $\Delta A_{Corrected}$ for each Sample.

$$\Delta A_{Corrected} = (A_{T2\ S} - A_{T1\ S}) - (A_{T2\ SBC} - A_{T1\ SBC})$$

6. Apply $\Delta A_{Corrected}$ to the Pyruvate Standard curve to get B nmol of Pyruvate generated by PEPCK at the reaction time ($\Delta T = T_2 - T_1$).
7. Calculate the PEPCK activity of the Sample:

   PEPCK Activity (nmol/min/µL or mU/µL) =

   $$[B/(\Delta T \times V)] \times D$$

where:

B = Pyruvate amount from Pyruvate Standard curve (pmol)

ΔT = Reaction time (T_2 - T_1) (minutes)

V = Sample volume added into the reaction well (µL)

D = Sample Dilution factor (if applicable; D = 1 for undiluted Samples)

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

Figure 1.
Typical Pyruvate Standard Curve.

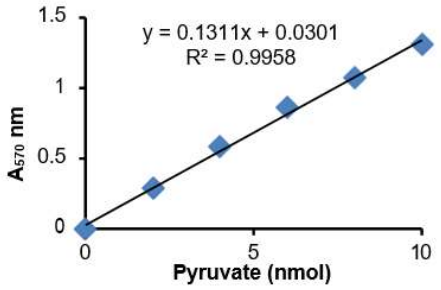


Figure 2.
Phosphoenolpyruvate Carboxykinase (PEPCK) kinetic activity measured in Lysates: HEK 293 cells (66 μg), Rat Liver (223 μg) and Rat Kidney (145 μg).

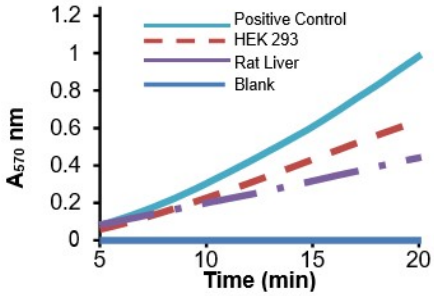
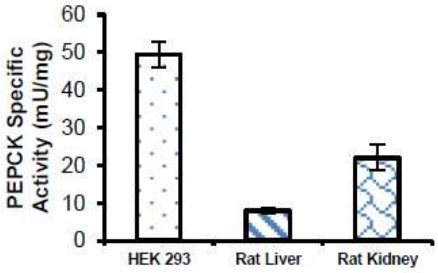


Figure 3.
PEPCK Specific Activity in biological samples. Assays were performed following kit protocol.



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