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ab239714
PEPCK Assay Kit (Phosphoenolpyruvate Carboxykinase)

For the detection of PEPCK activity in tissues, adherent cells and suspension cells.

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1. Overview

PEPCK Assay Kit (Phosphoenolpyruvate Carboxykinase) (ab239714) 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.

2. Protocol Summary

Prepare tissue or cell samples, background controls and positive controls.



Prepare standard curve.



Prepare standard mix, reaction mix and sample control mix and add to appropriate wells.



Measure the plate at OD 570nm in kinetic mode at 37°C for 10-60 mins.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 6 months from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
PEPCK Assay Buffer	25 mL	-20°C
OAA Enzyme Mix	1 vial	-20°C
Developer Mix A	1 vial	-20°C
PEPCK Positive Control	1 vial	-20°C
OxiRed™ Probe	0.2 mL	-20°C
PEPCK Substrate Mix	1 vial	-20°C
Pyruvate Standard	100 µL	-20°C

PLEASE NOTE: Developer Mix A was previously labelled as Developer V and PEPCK Developer, and OxiRed™ Probe as OxiRed Probe and PEPCK Probe (in DMSO). The composition has not changed.

5. Materials Required, Not Supplied

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

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer
- Dounce homogenizer
- 30% Glycerol

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 PEPCK Assay Buffer:

Ready to use as supplied. Warm to room temperature before use. Store at -20°C.

6.2 PEPCK Substrate, OAA Enzyme Mix and Developer Mix A:

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.

6.3 OxiRed™ Probe:

Ready to use as supplied. Warm the DMSO solution at 37 °C until completely thawed (~ 5 minutes), vortex briefly to create a homogenous solution, and spin down briefly to collect any contents trapped in the cap or seal. Keep at room temperature during use and reseal promptly to minimize moisture exposure.
For multiple use aliquot and store at −20 °C, protected from light and moisture. Stable for up to 2 months after thawing (≤3 freeze–thaw cycles).**PEPCK Positive Control:**

Reconstitute Reconstitute with 40 µl of 30% Glycerol. Store at -20°C. Keep on ice while in use. Use within two months.

6.4 Pyruvate Standard:

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

7. Standard Preparation

– Always prepare a fresh set of standards for every use.

7.1 Dilute Pyruvate Standard to 1 mM by taking 10 µl of 100 mM Pyruvate into 990 µl of PEPCK Assay Buffer, mix well.

7.2 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.

7.3 Adjust volume to 50 µl/well with PEPCK Assay Buffer, mix well.

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

8. Sample Preparation

8.1 Homogenize tissue (20 mg) or cells (2 x 10^6) with 200 µl ice cold Assay Buffer for 10 mins on ice.

8.2 Centrifuge at 10,000 *X g* at 4°C for 10 mins.

8.3 Collect the supernatant and measure the protein concentration.

8.4 Add 2-50 µl sample per well, adjust final volume to 50 µl with Assay Buffer.

8.5 For positive control, add 2-10 µl of reconstituted PEPCK positive control, and adjust final volume to 50 µl with Assay Buffer.

8.6 For 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.

Δ Note: For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

9. Assay Procedure

9.1 Make enough reagents for the number of assays to be performed.

9.2 For each well, prepare 50 µl Reaction Mix containing:

Component	Standard Mix µL	Reaction Mix µL	Sample Control Mix µL
PEPCK Assay Buffer	46	42	44
OAA Enzyme Mix	---	2	2
Developer Mix A	2	2	2
OxiRed™ Probe	2	2	2
PEPCK Substrate Mix	---	2	---

9.3 Mix well, then add 50 µl of the Standard Mix to Pyruvate Standard Curve; Add 50 µl of the Reaction Mix to each well containing test samples.
Δ Note: For samples having high background, add 50 µl of Sample Control Mix to sample background control well(s). Mix well.

9.4 Measure the plate at OD 570nm in kinetic mode at 37°C for 10-60 mins. We recommend measuring the absorbance in kinetic mode, and choosing two time points (t1 and t2) 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).

10. Data Analysis

10.1 Subtract the 0 standard reading from all readings.
10.2 Plot the Pyruvate standard curve.
10.3 If the sample background is high, subtract the background control reading from sample reading.
10.4 Calculate the PEPCK activity of the test sample.
10.5 Determine the ΔOD (ΔOD = OD2- OD1) at linear range of two time point (t1 and t2), apply the ΔOD to the Pyruvate standard curve to get B nmol of Pyruvate generated by PEPCK at the reaction time (Δt = t2-t1).

Sample PEPCK Activity =

$$\frac{B}{(\Delta T \; x \; V)} x \; D$$

= nmol/min/µL = mU/µL.

Where:

B is Pyruvate amount from the standard curve (nmol).
V is sample volume added into the reaction well (µl).
T is the time (mins).
D is the 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.

11. Typical Data

Typical data provided **for demonstration purposes only**.

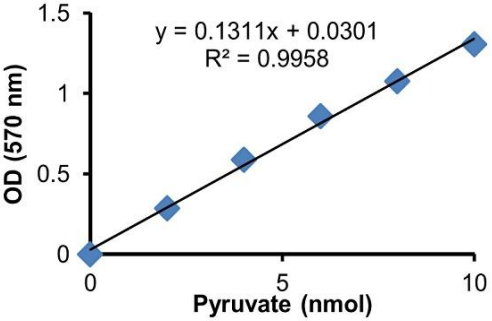


Figure 1. Pyruvate standard curve.

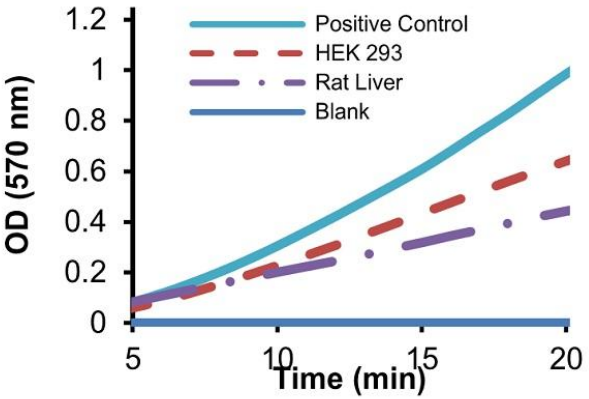


Figure 2. Phosphoenolpyruvate Carboxykinase (PEPCK) kinetic activity measured in lysates: HEK-293 cells (66 µg), rat liver (223 µg) and rat kidney (145 µg).

Typical data provided **for demonstration purposes only**.

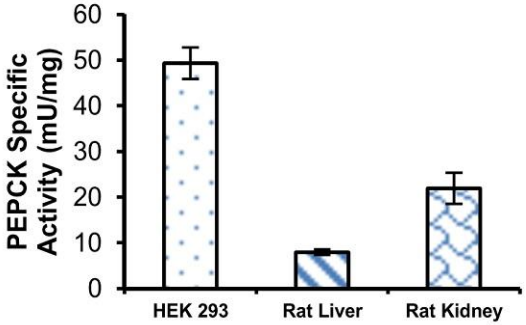


Figure 3. PEPCK Specific Activity in biological samples.

12. Notes

Technical Support

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