Version 3a, Last updated 6 June 2025

# ab204713 Phosphoenolpyruvic acid (PEP) Assay Kit

For the measurement of PEP levels in various samples.

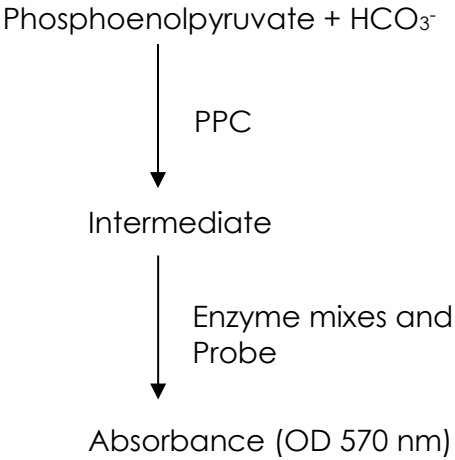
This product is for research use only and is not intended for diagnostic use.

## Table of Contents

# 1. Overview

Phosphoenolpyruvic acid (PEP) Assay Kit (ab204713) provides a convenient colorimetric and fluorometric means to measure PEP levels in various samples. In the assay, PEP is converted to ATP and pyruvate. The generated pyruvate is quantified by colorimetric (λmax = 570 nm) or fluorometric methods (Ex/Em = 535/587 nm). The assay is simple, sensitive and reliable. The detection limit is approximately 1 µM PEP in biological samples.

## 2. Protocol Summary



## 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.

## 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 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature (see section 6).

| Item | Quantity | Storage condition |
|---|---|---|
| Assay Buffer 4 | 25 mL | -20°C |
| OxiRed™ Probe | 0.2 mL | -20°C |
| PEP | 1 vial | -20°C |
| PEP Converter Mix | 1 vial | -20°C |
| Developer Mix A | 1 vial | -20°C |

PLEASE NOTE: Assay Buffer 4 was previously labelled as Assay Buffer IV and PEP Assay Buffer, and OxiRed™ Probe as OxiRed Probe and PEP Probe (in DMSO). Developer Mix A was previously labelled as Development Enzyme Mix I and PEP Developer Mix. 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:

- 3M $HClO_4$
- 3M $KHCO_3$
- activated charcoal

## 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 Assay Buffer 4:

Bring to room temperature before use.

### 6.2 OxiRed™ Probe:

Ready to use as supplied. Warm for 1-2 min at 37°C to melt the frozen DMSO before use. Mix well. Store at –20°C, protect from light and moisture. Use within two months.

### 6.3 PEP:

Dissolve in 100 µL $dH_2O$ to generate 10 mM stock solution. Keep cold while in use. Store at -20°C.

### 6.4 PEP Converter Mix:

Dissolve with 220 µL Assay Buffer 4. Pipette up and down to dissolve. Store at –20°C. Use within two months.

### 6.5 Developer Mix A:

Dissolve with 220 µL Assay Buffer 4. Pipette up and down to dissolve. Store at –20°C. Use within two months.

## 7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

### 7.1 For Colorimetric Assay:

7.1.1 Prepare 1 mM (1 nmol/uL) PEP Assay Standard by adding 10 µL of the 10 mM Standard to 90µL of Assay Buffer 4, mix well.
7.1.2 Add 0, 2, 4, 6, 8, 10 µL into a series of wells.
7.1.3 Adjust volume to 50 µL/well with Assay Buffer 4 to generate 0, 2, 4, 6, 8, 10 nmol/well of PEP.

| Standard # | 1 mM PEP Assay Standard (µL) | Assay Buffer 4 (µL) | PEP nmoles/well |
|---|---|---|---|
| 1 | 10 | 40 | 10 |
| 2 | 8 | 42 | 8 |
| 3 | 6 | 44 | 6 |
| 4 | 4 | 46 | 4 |
| 5 | 2 | 48 | 2 |
| 6 | 0 | 50 | 0 |

### 7.2 For Fluorometric Assay:

7.2.1 Prepare 0.1 mM (0.1 nmol/µL) PEP Assay Standard by adding 1 µL of the 10 mM Standard to 99 µL of Assay Buffer 4, mix well (the fluorometric assay is 10 to 100 fold more sensitive than the colorimetric assay).

7.2.2 Add 0, 2, 4, 6, 8, 10 µL into a series of standards wells.

7.2.3 Adjust volume to 50 µL/well with Assay Buffer 4 to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well of PEP.

| Standard # | 0.1 mM PEP Assay Standard (µL) | Assay Buffer 4 (µL) | PEP nmoles/well |
|---|---|---|---|
| 1 | 10 | 40 | 1.0 |
| 2 | 8 | 42 | 0.8 |
| 3 | 6 | 44 | 0.6 |
| 4 | 4 | 46 | 0.4 |
| 5 | 2 | 48 | 0.2 |
| 6 | 0 | 50 | 0 |

## 8. Sample Preparation

**8.1** Tissue (20-50 mg) should be frozen in liquid $N_2$ or dry ice/MeOH then powdered thoroughly with mortar and pestle at -80°C and transferred to an Eppendorf tube.

**8.2** Add 100 µL ice cold $HClO_4$ and vortex until the contents are thoroughly mixed.

**8.3** Neutralize carefully by adding repeated small aliquots (~10 µL per aliquot) of $KHCO_3$ followed by vortexing. Final pH should be 6.5-7.5.

**8.4** Centrifuge at 12,000 *g* for 3 min.

**Δ Note:** Some plant extracts need to be decolorized with activated charcoal (5 mg per tube) which can be added and vortexed prior to the centrifugation step.

**8.5** Use samples immediately or store at -80°C.

**8.6** Add up to 50 µL sample per well in a 96-well plate; bring the volume to 50 µL with Assay Buffer 4.

**Δ Note:** Intracellular PEP level is usually in the range of 0.05 - 0.3 mM. We suggest testing several doses of your sample to ensure the readings are within the standard curve range.

## 9. Assay Procedure

**9.1** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µL mix containing:

| | Reaction Mix | |
|---|---|---|
| | **Colorimetric Assay** | **Fluorometric Assay** |
| Assay Buffer 4 | 44 µL | 45.8 µL |
| OxiRed™ Probe | 2 µL | 0.2 µL* |
| PEP Converter Mix ** | 2 µL | 2 µL |
| Developer Mix A | 2 µL | 2 µL |

**9.2** Mix and add 50 µL of the Reaction Mix to each well containing the Standard Curve and test samples.

**9.3** Incubate at room temperature for 1 h, protected from light.

**9.4** Measure O.D. 570 nm for colorimetric assay or Ex/Em 535/587 nm for fluorometric assay.

**Δ Note:**

- *For the fluorometric assay, use 1/10 of OxiRed™ Probe to reduce fluorescence background.
- **Pyruvate generates background.
    - If significant amount of pyruvate is suspected in your samples, a sample pyruvate background control needs to be performed by replacing the PEP Converter Mix with 2 µL of Assay Buffer 4.
    - Then follow the same protocol as the sample.
    - In the absence of PEP Converter Mix, the assay detects only pyruvate, not PEP. The pyruvate background reading can be subtracted from the PEP readings.

## 10. Calculations

**10.1** Subtract 0 PEP control reading from all readings. Plot the PEP Assay Standard Curve.

**10.2** If a sample pyruvate background control was used, subtract the background control reading from the PEP readings.

**10.3** Apply the sample readings to the standard curve to get PEP

**10.4** amount in the sample wells.

**10.5** The PEP concentrations in the test samples:

C = Ay/Sv (nmol/ µL, µmol/mL or mM)

Where:

Ay is the PEP amount (nmol) in your sample from the standard curve.

Sv is the sample volume (µL) added to the assay well.

PEP molecular weight: 168.04.

## 11. Typical Data

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

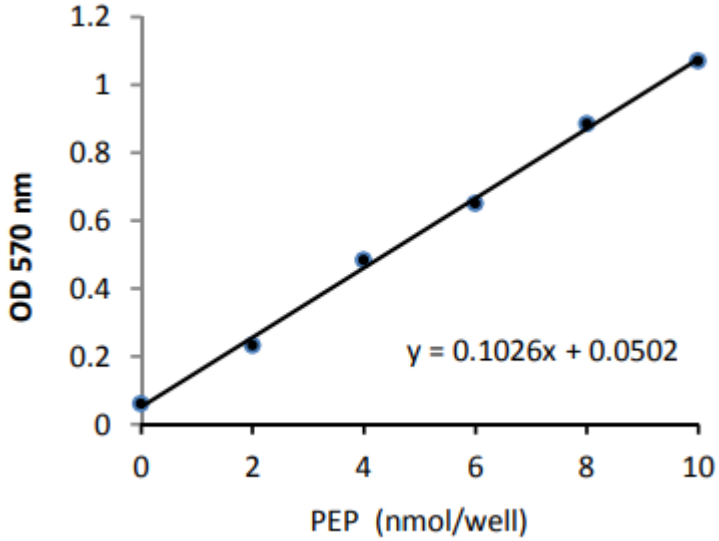



**Figure 1**. Colorimetric Assay standard curve.

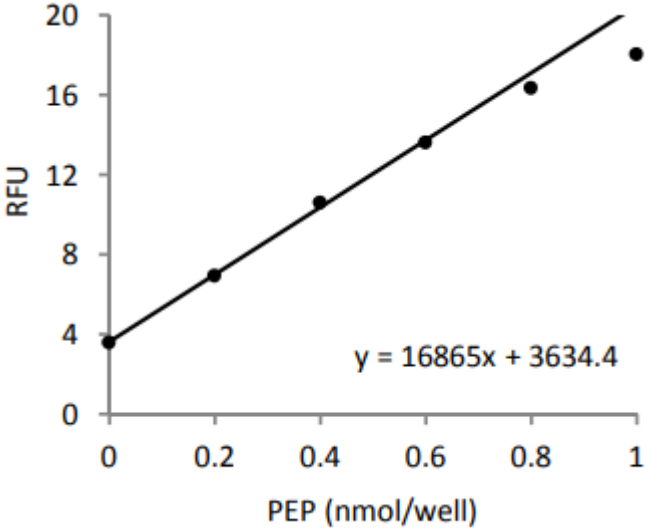



**Figure 2.** Fluorometric Assay standard curve.

## 12. Notes

**Technical Support**



For all technical or commercial enquiries please go to:
www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)