BioVision

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For research use only

# PEP Colorimetric/Fluorometric Assay Kit

(Catalog# K365-100; 100 Assays; Store at -20°C)

## I. Introduction:

Phosphoenolpyruvate (PEP) is an important intermediate in carbohydrate metabolism. Containing a high-energy phosphate bond, PEP is involved in glycolysis and gluco-neogenesis and in the shikimate pathway as well as in carbon fixation in plants. Bacteria utilize PEP in the phosphor-transferase system used acquire sugars from the environment. In the glycolytic pathway, PEP is formed from 2-phosphoglycerate by enolase and generates ATP through the action of pyruvate kinase. BioVision's PEP Assay Kit provides a convenient colorimetric and fluorometric means to measure PEP levels. In the assay, PEP is converted to ATP and pyruvate. The generated pyruvate is quantified by colorimetric ($\lambda_{max}$ = 570 nm) or fluorometric methods (Ex/Em 535/587 nm). The assay is simple, sensitive and stable. The detection limit is approximately 1 µM PEP in biological samples.

## II. Kit Contents:

| Components | K365-100 | Cap Code | Part Number |
|---|---|---|---|
| PEP Assay Buffer | 25 ml | WM | K365-100-1 |
| PEP Probe (in DMSO) | 0.2 ml | Red | K365-100-2A |
| PEP Converter | 1 vial | Purple | K365-100-4 |
| PEP Developer Mix | 1 vial | Green | K365-100-5 |
| PEP Standard (1 µmole) | 1 vial | Yellow | K365-100-6 |

## III. Storage and Handling:

Store kit at –20°C, protect from light. Warm the PEP Assay Buffer to room temperature prior to use. Briefly centrifuge all vials prior to opening. Read the entire protocol before performing the assay. **Not included but needed: liquid N2 or dry/ice methanol, 3M $HClO_4$, 3M $KHCO_3$, activated charcoal.**

## IV. Reagent Preparation and Storage Conditions:

**PEP Probe:** Ready to use as supplied. Warm 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.

**PEP Converter and PEP Developer Mix:** Dissolve with 220 µl PEP Assay Buffer separately. Pipette up and down to dissolve. Store at –20°C. Use within two months.

**PEP Standard:** Dissolve in 100 µl $dH_2O$ to generate 10 mM stock solution. Keep cold while in use. Store at -20°C.

## V. PEP Assay Protocol:

1. **Standard Curve Preparations:**
**For Colorimetric Assay:** Dilute the PEP Standard to 1 nmol/µl by adding 10 µl of the 10 mM Standard to 90µl of PEP Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells. Adjust volume to 50 µl/well with PEP Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of PEP Standard.
**For Fluorometric Assay:** Dilute the PEP Standard to 0.1 nmol/µl (the fluorometric assay is 10 to 100 fold more sensitive than the colorimetric assay). Add 0, 2, 4, 6, 8, 10 µl into a series of standards wells. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well of PEP standard.
2. **Sample Preparation:** 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. Add 100 µl ice cold $HClO_4$ and vortex until the contents are thoroughly mixed. 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. Centrifuge at 12,000g for 3 minutes. 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. Use samples immediately or store at -80°C. Add up to 50 µl sample per well in a 96-well plate; bring the volume to 50 µl with Assay Buffer.
3. 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.
4. **PEP Reaction Mix:** Mix enough reagent for the number of samples and standards to be performed: For each well, prepare a total 50 µl Reaction Mix containing:

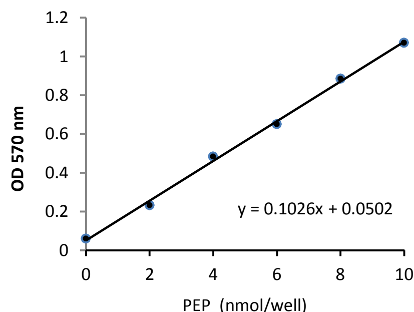
| | Colorimetric Assay | Fluorometric Assay |
|---|---|---|
| PEP Assay Buffer | 44 µl | 45.8 µl |
| PEP Probe | 2 µl | 0.2 µl* |
| PEP Converter** | 2 µl | 2 µl |
| PEP Developer | 2 µl | 2 µl |

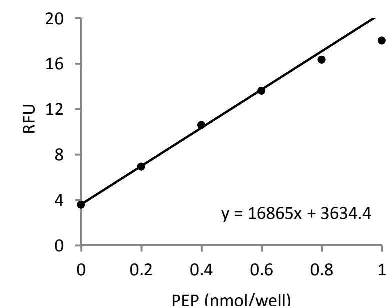
**Notes:**
*For the fluorometric assay, use 1/10 of PEP probe to reduce fluorescence background.
**Pyruvate generates background. If significant amount of pyruvate is suspected in your samples, a sample pyruvate background control needs to be performed by replacing the PEP Converter with 2 µl of assay buffer. Then follow the same protocol as the sample. In the absence of PEP converter, the assay detects only pyruvate, not PEP. The pyruvate background reading can be subtracted from the PEP readings.

5. Add 50 µl of the Reaction Mix to each well containing the PEP Standard and test samples. Incubate at room temperature for 1 hr, protected from light.
6. Measure O.D. 570 nm for colorimetric assay or Ex/Em 535/587 nm for fluorometric assay.
7. **Calculation:** Subtracting the 0 PEP control from all standard and sample readings (Note: The background reading can be significant and must be subtracted from sample readings). Plot PEP standard curve. Apply the sample readings to the standard curve to get PEP amount in the sample wells. 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.




**RELATED PRODUCTS:**

- Apoptosis Detection Kits & Reagents
- Glucose, Sucrose, Glycogen Assay Kit
- Glutathione Assay Kit
- NAD/NADH and NPEP/NPEPH Assay Kits
- ATP Fluorometric/Colorimetric Assay Kit
- Ascorbic Acid Assay kit
- Cell Proliferation & Senescence Kits
- Cholesterol, LDL/HDL, Triglyceride Assay Kits
- Ethanol, Fatty Acid and Uric Acid Assay Kits
- Glucose and Lactate Assay Kits
- Pyruvate Assay Kit
- Amino Acid Assay Kits

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

## GENERAL TROUBLESHOOTING GUIDE:

| Problems | Cause | Solution |
|---|---|---|
| Assay not working | • Use of ice-cold reaction buffer<br>• Omission of a step in the protocol<br>• Plate read at incorrect wavelength<br>• Use of a different 96-well plate | • Reaction buffer must be at room temperature<br>• Refer and follow the data sheet precisely<br>• Check the wavelength in the data sheet and the filter settings of the instrument<br>• Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates |
| Samples with erratic readings | • Use of an incompatible sample type<br>• Samples prepared in a different buffer<br>• Samples were not deproteinized (if indicated in datasheet)<br>• Cell/ tissue samples were not completely homogenized<br>• Samples used after multiple free-thaw cycles<br>• Presence of interfering substance in the sample<br>• Use of old or inappropriately stored samples | • Refer data sheet for details about incompatible samples<br>• Use the reaction buffer provided in the kit or refer data sheet for instructions<br>• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated<br>• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope<br>• Aliquot and freeze samples if needed to use multiple times<br>• Troubleshoot if needed, deproteinize samples<br>• Use fresh samples or store at correct temperatures till use |
| Lower/ Higher readings in Samples and Standards | • Improperly thawed components<br>• Use of expired kit or improperly stored reagents<br>• Allowing the reagents to sit for extended times on ice<br>• Incorrect incubation times or temperatures<br>• Incorrect volumes used | • Thaw all components completely and mix gently before use<br>• Always check the expiry date and store the components appropriately<br>• Always thaw and prepare fresh reaction mix before use<br>• Refer datasheet & verify correct incubation times and temperatures<br>• Use calibrated pipettes and aliquot correctly |
| Readings do not follow a linear pattern for Standard curve | • Use of partially thawed components<br>• Pipetting errors in the standard<br>• Pipetting errors in the reaction mix<br>• Air bubbles formed in well<br>• Standard stock is at an incorrect concentration<br>• Calculation errors<br>• Substituting reagents from older kits/ lots | • Thaw and resuspend all components before preparing the reaction mix<br>• Avoid pipetting small volumes<br>• Prepare a master reaction mix whenever possible<br>• Pipette gently against the wall of the tubes<br>• Always refer the dilutions in the data sheet<br>• Recheck calculations after referring the data sheet<br>• Use fresh components from the same kit |
| Unanticipated results | • Measured at incorrect wavelength<br>• Samples contain interfering substances<br>• Use of incompatible sample type<br>• Sample readings above/below the linear range | • Check the equipment and the filter setting<br>• Troubleshoot if it interferes with the kit<br>• Refer data sheet to check if sample is compatible with the kit or optimization is needed<br>• Concentrate/ Dilute sample so as to be in the linear range |
| **Note:** The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems. | | |