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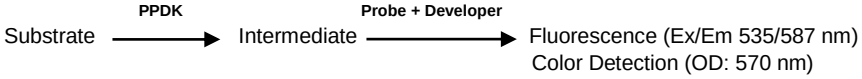
PPDK (Pyruvate, phosphate dikinase) Activity Assay Kit (Fluorometric/Colorimetric)

rev 09/19

(Catalog #K456-100; 100 assays; Store kit at -20°C)

I. Introduction:

Pyruvate, phosphate dikinase, PPDK, (E.C. 2.7.9.1) is found in microorganisms and plants where it catalyzes reversible interconversion of AMP, PPi and phosphoenolpyruvate (PEP) to ATP, Pi and pyruvate monophosphate. PPDK plays an important role in plant photosynthesis and glycolytic pathway of endobiotic protozoa and bacteria where pyruvate, phosphate dikinase (PK) is absent; it functions in the process of ATP biosynthesis. In plants, PPDK is a pivotal regulator of the C4 photosynthetic cycle and the key limiting factor for maintaining photosynthesis at low temperature. It catalyzes the regeneration of PEP as the primary acceptor of atmospheric CO_2 from pyruvate, ATP and phosphate. In addition, PPDK is also present in C3 leaves where it confers a similar light/dark regulation in chloroplasts, and in wheat leaves in an amount comparable to that found in seeds. Since PPDK is not found in vertebrates, finding its inhibitors may be useful for treatment of infections caused by anaerobic protists that depend on pyrophosphate-dependent glycolysis. Specific inhibition of PPDK is expected to inhibit *Giardia* and other anaerobic protists such as *Entamoeba histolytica* and *Trichomonas vaginalis* without harming their mammalian hosts. BioVision's Pyruvate, phosphate dikinase, (PPDK) Assay Kit provides a simple and rapid test for measuring specific activity of PPDK in bacterial, protozoan and plant lysates as well as protein preps. We utilize the ability of PPDK to interconvert the substrate into intermediate product detected by proprietary enzyme mix and probe. Generated stable product can be quantified by either colorimetric or fluorometric readout. The assay is simple to perform, high-throughput adaptable and a fluorometric reaction can detect as low as of 0.006 U of PPDK activity in a single well. We provide sufficient reagents for 100 fluorometric or colorimetric assays.



II. Applications:

- Measurement of PPDK activity in tissue/cell lysates and protein preparations
- Screening for specific inhibitors of PPDK activity that may affect microorganisms/plant biochemical pathways
- Study of pyrophosphate-mediated glycolysis in bacterial and protozoan cell lysates
- Detection of activity and light activation kinetics of PPDK from crude leaf extracts and recombinant protein
- Study of photosynthesis in plants

III. Sample Type:

- Tissue culture extracts and lysates; protein preps

IV. Kit Contents:

Components	K456-100	Cap Code	Part Number
PPDK Assay Buffer	25 ml	WM	K456-100-1
PPDK Cofactor Mix	200 µl	Clear	K456-100-2
PPDK Substrate Mix	1 vial	Orange	K456-100-3
PPDK Positive Control	50 µl	Blue	K456-100-4
PPDK Developer	1 vial	Green	K456-100-5
PPDK Probe	200 µl	Red	K456-100-6
Pyruvate Standard (100 mM)	100 µl	Yellow	K456-100-7

V. User Supplied Reagents and Equipment:

- 96-well clear plate
- Multi-well spectrophotometer

VI. Storage Conditions and Reagent Preparation:

Upon receiving store the entire kit at -20°C protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **PPDK Cofactor Mix:** Thaw and keep on ice while in use. Use within one month.
- **PPDK Positive Control:** Store at -20°C, use within one month.
- **PPDK Substrate Mix:** Dissolve with 200 µl of ddH_2O and store at -20°C. Use within one month.
- **PPDK Developer:** Dissolve with 220 µl of PPDK Assay Buffer, aliquot and store at -20°C, protect from light. Use within one month.
- **PPDK Assay Buffer, PPDK Probe and Standard:** Warm to RT before use. Store at -20°C. Use within one month.

VII. PPDK Activity Assay Protocol:

1. **Sample Preparation:** Samples from tissue or cell cultures can be prepared by your method of choice or extracted directly in 10 volumes of ice cold PPDK Assay Buffer (i.e. 10 mg sample/100 µl Assay Buffer). Centrifuge the samples (10K x g; 10 min.; 4°C) to remove insoluble material and collect the supernatant. **Optionally:** concentrate pre-cleared supernatant through a 10 KDa spin column (10K x g at 4°C, 10 min; BV #1997) and measure the amount of protein in the ultraconcentrate sample using BCA Protein Assay Kit (**Cat. K818-1000, K819-250** or equivalent). Add 2-50 µl of sample into a 96-well plate and adjust the volume to 50 µl with PPDK Assay Buffer. Prepare positive control well(s) by adding 2 µl of PPDK Positive Control and adjust the volume to 50 µl with PPDK Assay Buffer. For samples exhibiting significant background, prepare background control well(s) containing the same sample volumes as the sample wells.
2. **Standard Curve Preparation:** For **colorimetric** assay: dilute the Standard to 1 nmol/µl by adding 10 µl of the 100 nmol/ µl Standard to 990 µl of PPPDK Assay Buffer, mix well. For **fluorometric** assay: Dilute the Standard to 1 nmol/µl as for the colorimetric assay, then further dilute the 1 nmol/µl Standard to 0.1 nmol/µl by adding 10 µl of 1 nmol/µl Standard into 90 µl of PPDK Assay Buffer, mix well. Add

0, 2, 4, 6, 8, 10 µl of the diluted Standard into a series of wells of a 96-well plate. Adjust the volume to 50 µl/well with PPDK Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Standard for the colorimetric assay or 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well for the fluorometric assay respectively.

3. **Reaction Mix:** Prepare enough reaction mix for the number of assays to be performed according to the table below. Mix well and add 50 µl of Reaction Mix to their respective wells.

Reaction Mix	Standard Curve	[a] Sample & Positive Control	Background Control Mix
PPDK Assay Buffer	46 µl	42 µl	44 µl
PPDK Cofactor Mix	-	2 µl	2 µl
PPDK Substrate	-	2 µl	-
PPDK Developer	2 µl	2 µl	2 µl
PPDK Probe	2 µl	2 µl	2 µl

Notes: [a] Do not add PPDK Positive Control to the Sample wells.

4. **Measurement:** Measure **absorbance** (570 nm) or **fluorescence** (Ex/Em = 535/587 nm) of the samples and controls in kinetic mode for 60 min. at 37°C. The Pyruvate Standard Curve can be read in endpoint mode after 60 min the incubation time.
5. **Calculations**: Subtract 0 Standard reading from all the standard readings, plot the Standard Curve and calculate the slope. For sample and background control wells: choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values. Determine changes in fluorescence or absorbance over the time interval (ΔRFU or ΔOD) and substrate the background control values from each sample. Apply the corrected fluorescence or absorbance values to Standard Curve to obtain corresponding nmol of product formed during the reaction time (**B**, in nmol).

$$\textbf{Sample PPDK Activity} = B/(\Delta t \times V) \times \textbf{Dilution Factor} = \textbf{nmol/min/ml} = \textbf{mU/ml}$$

Where: **B** = nmol of product formed calculated from Standard Curve (nmol)
V = Sample volume added into the reaction well (ml)
Δt = reaction time (min)

PPDK Unit Definition: 1 unit is defined as the amount of PPDK that will generate 1 µmol of product per min at 37°C and pH 7.

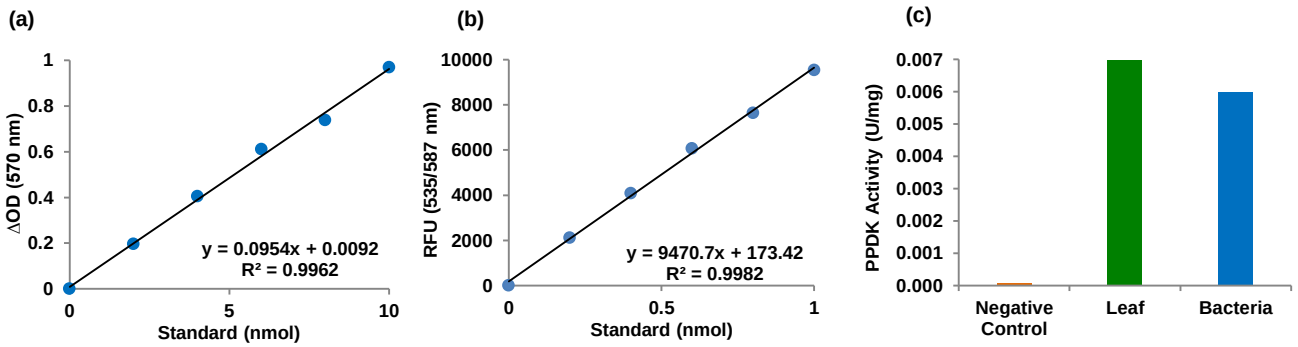


Figure: Standard Curves: Colorimetric (a) and Fluorometric (b). Quantification of PPDK Activity in protein extracts; 40 µg of Leaf; 4.8 µg of *E. coli* expressing PPDK; 120 µg of Negative Control (untransformed *E. coli* strain). Activity was calculated according to the kit protocol using colorimetric method.

VIII. RELATED PRODUCTS:

Pyrophosphate (PPi) Assay Kit (Fluorometric/Colorimetric) (K568)
PPDK, Active, *M. rosea* Recombinant (P1161)
Phosphate Assay Kit (Fluorometric) (K420)
ATP Colorimetric/Fluorometric Assay Kit (K354)
Phosphate Colorimetric Assay Kit (K410)
DNAse I Activity Assay Kit (Fluorometric) (K429)
EZScreen™ ATP Colorimetric Assay Kit (384-well) (K959)
StayBrite™ Highly Stable ATP Bioluminescence Assay Kit (K791)
ApoSENSOR™ ATP Cell Viability Bioluminescence Assay Kit (K254)
ADP/ATP Ratio Bioluminescence Assay Kit, ApoSENSOR (K255)
S-adenosylmethionine synthetase (AdoMetS), Active, *E. coli* Recombinant (P1112)

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