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ab273318 Pyruvate, phosphate dikinase (PPDK) Activity Assay Kit (Fluorometric/ Colorimetric)

View Kit datasheet: https://www.abcam.com/ab273318
(use https://www.abcam.cn/ab273318 for china, or
https://www.abcam.co.jp/ab273318 for Japan)

For the determination of Pyruvate, phosphate dikinase (PPDK) activity in tissue culture extracts/lysates and protein preparations.

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

Table of Contents

1. Overview

Pyruvate, phosphate dikinase (PPDK) Activity Assay Kit (Fluorometric) (ab273318) 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.

2. Protocol Summary

Prepare samples and positive control as directed.

Prepare all reagents as directed.

Prepare standards for colorimetric assay or fluorometric assay as directed.

Add Positive Control, Samples, Background Control to appropriate wells and adjust volume to 50 µL

Add Standard Curve Mix, Reaction Mix or Background Control Mix (50 µL)

Measure absorbance (570 nm) or fluorescence (Ex/Em= 535/587 nm) of the samples and controls in kinetic mode for 60 minutes at 37°C.

Pyruvate Standard Curve can be read in endpoint mode after 60 minutes the incubation time.

Calculate PPDK activity using equation

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 month from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature
PPDK Assay Buffer	25 mL	-20°C
PPDK Cofactor Mix	200 µL	-20°C
PPDK Substrate Mix (Lyophilized)	1 vial	-20°C
PPDK Positive Control	50 µL	-20°C
PPDK Developer (Lyophilized)	1 vial	-20°C
PPDK Probe	200 µL	-20°C
Pyruvate Standard	100 µL	-20°C

7. Materials Required, Not Supplied

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

- Multi-well spectrophotometer
- 96-well clear plate with flat bottom

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 PPDK Assay Buffer:

Warm to room temperature before use.
Store at -20 °C.
Use within one month.

9.2 PPDK Cofactor Mix:

Thaw and keep on ice while in use.
Use within one month.

9.3 PPDK Substrate Mix (Lyophilized):

Reconstitute with 200 µl ddH_2O.
Aliquot and store at -20 °C.
Use within one month.

9.4 PPDK Positive Control:

Store at -20 °C.
Use within one month.

9.5 PPDK Developer (Lyophilized):

Reconstitute with 220 µl PPDK Assay Buffer.
Aliquot and store at -20 °C, protect from light.
Use within one month.

9.6 PPDK Probe:

Warm to room temperature before use.
Store at -20 °C.
Use within one month.

9.7 Pyruvate Standard (100 mM):

Warm to room temperature before use.
Store at -20 °C.
Use within one month.

10. Sample Preparation

Tissue and cell preparation:

10.1 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).

10.2 Centrifuge the samples (10,000 *x g*; 10 mins at 4°C) to remove insoluble material and collect the supernatant.

Optionally: concentrate pre-cleared supernatant through a 10 KDa spin column (10,000 *x g* at 4°C, 10 mins) and measure the amount of protein in the ultra-concentrate sample using a BCA Protein Assay Kit or equivalent.

11. Standard Curve

11.1 Colorimetric assay:

11.1.1 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.

11.1.2 Add 0, 2, 4, 6, 8, 10 µl of the diluted Standard into a series of wells of a 96-well plate and adjust the volume to 50 µl/well with PPDK Assay Buffer. This will generate 0, 2, 4, 6, 8, 10 nmol/well of the Standard respectively.

Standard #	1 nmol/µl Pyruvate Standard (µL)	PPDK Assay Buffer (µL)	Pyruvate (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

11.2 Fluorometric assay:

11.2.1 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.

11.2.2 Add 0, 2, 4, 6, 8, 10 µl of the diluted Standard into a series of wells of a 96-well plate and adjust the volume to 50 µl/well with PPDK Assay Buffer. This will generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well the Standard respectively.

Standard #	0.1 nmol/µl Pyruvate Standard (µL)	PPDK Assay Buffer (µL)	Pyruvate (nmol/well)
1	0	50	0
2	2	48	0.2
3	4	46	0.4
4	6	44	0.6
5	8	42	0.8
6	10	40	1.0

12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

12.1 Add 2-50 µl of sample into a 96-well plate and adjust the volume to 50 µl with PPDK Assay Buffer.

12.2 For Positive Control, add 2 µl of PPDK Positive Control to desired wells and adjust the volume to 50 µl with PPDK Assay Buffer.

12.3 For samples exhibiting significant background, prepare background control wells containing the same sample volumes as the sample wells.

Reaction Mix:

12.4 Prepare enough reagents for the number of assays to be performed. For each well, prepare 50 µl of appropriate Mix:

Component	Standard Curve Mix (µL)	Reaction Mix (µL)	Background Control Mix (µL)
PPDK Assay Buffer	46	42	44
PPDK Cofactor Mix	---	2	2
PPDK Substrate	---	2	---
PPDK Developer	2	2	2
PPDK Probe	2	2	2

∆ **Note:** Do not add PPDK Positive Control to the Sample wells.

12.5 Mix and add 50 µl of the Standard Curve Mix to wells containing Standard Curve.

12.6 Add 50 µl of the Reaction Mix to wells containing Positive Control and Samples.

12.7 Add 50 µl of the Background Control Mix to wells containing Sample Background Control.

12.8 Measure absorbance (570 nm) or fluorescence (Ex/Em = 535/587 nm) of the samples and controls in kinetic mode for 60 minutes at 37°C.

Δ Note: Pyruvate Standard Curve can be read in endpoint mode after 60 minutes the incubation time.

13. Calculations

13.1 Subtract 0 Standard reading from all Standard readings.
13.2 Plot the Standard Curve and calculate the slope.
For sample and background control wells:
13.3 Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values.
13.4 Determine changes in fluorescence or absorbance over the time interval (ΔRFU or ΔOD) and substrate the background control values from each sample.
13.5 Apply the corrected fluorescence or absorbance values to Standard Curve to obtain corresponding nmol of product formed during the reaction time (B, in nmol).
13.6 Calculate the PPDK activity of the test samples:

$$Sample\ PPDK\ activity\ = \frac{B}{(\Delta t\ x\ V)} x\ D \quad (pmol/min/ml)$$

B = Product formed calculated from Standard Curve (nmol)
Δt = Reaction time (min)
V = Sample volume added into the reaction well (ml)
D = Dilution factor (D = 1 for undiluted Samples)

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.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

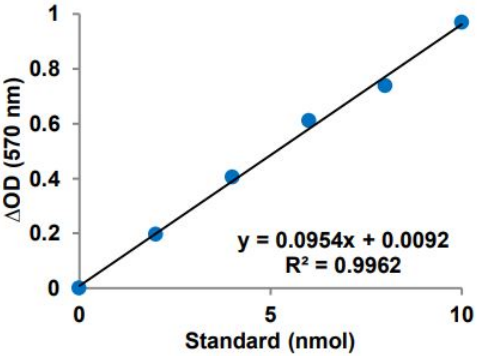

Figure 1. Standard Curve: Colorimetric.

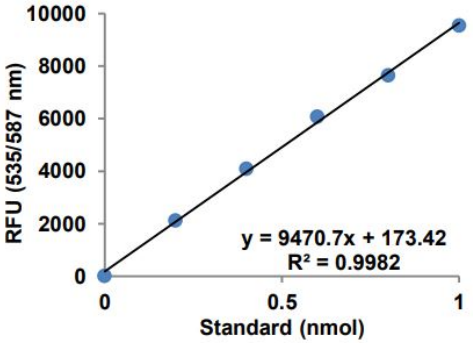

Figure 2. Standard Curve: Fluorometric.

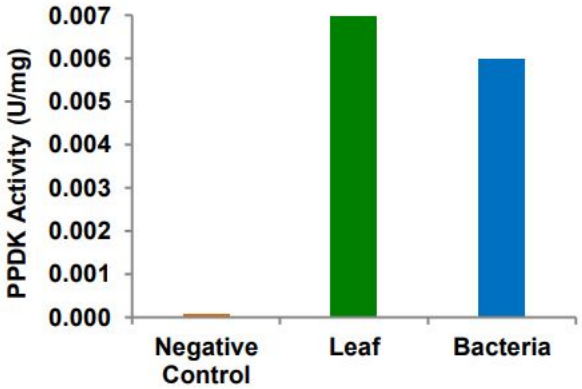

Figure 3. Quantification of PPDK Activity in protein extracts; 40 µg of Leaf; 4.8 µg of E. coli expressing PPDK; 120 µg of Negative Control (untransformed E. coli strain). Activity was calculated according to the kit protocol using colorimetric method.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. 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)