EnzyChrom™ Pyruvate Kinase Kit (EPRK-100)

Colorimetric/Fluorimetric Determination of Pyruvate Kinase Activity

DESCRIPTION

PYRUVATE KINASE (PK) is an enzyme involved in glycolysis. It catalyzes the transfer of a phosphate group from phosphoenolpyruvate (PEP) to ADP, yielding one molecule of pyruvate and one molecule of ATP. Pyruvate kinase deficiency, a genetic disease, is caused by a lack of pyruvate kinase and slows down the process of glycolysis. Pyruvate kinase is also involved in gluconeogenesis, a biochemical pathway in which the liver generates glucose from pyruvate and other substrates.

BioAssay Systems' Pyruvate Kinase Assay Kit provides a simple, direct and automation-ready procedure for measuring pyruvate kinase activity. In this assay PEP and ADP are catalyzed by pyruvate kinase to generate pyruvate and ATP. The color intensity of the reaction product at 570nm or fluorescence intensity at λex/em = 530/590nm is directly proportional to the pyruvate generated by the Pyruvate Kinase in the sample.

KEY FEATURES

Sensitive and accurate. Linear detection range in 96-well plate: 0.1 to 50 U/L for colorimetric assays and 0.01 to 2 U/L for fluorimetric assays run at 25°C for 30 min.

APPLICATIONS

Direct Assays: PK activity levels in plasma, serum, and tissue samples.

Drug Discovery/Pharmacology: effects of drugs on PK activity.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Developer:	12 mL	**Cosubstrate:**	120 µL
Dye Reagent:	120 µL	**Pyruvate Standard:**	400 µL 25 mM

Storage conditions. The kit is shipped on ice. Store all kit components at -20 °C. Shelf life of six months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Important:: equilibrate Developer to desired assay temperature.

Sample Preparation

Tissue or cell samples (2×10^6) can be homogenized in 100 µL PBS. Centrifuge at 14,000 rpm for 5 min. Use clear supernatant for assay.

Serum should be diluted at least 4-fold in dH_2O.

Colorimetric Procedure

1. *Standards*. First dilute the Pyruvate Standard to 1000 µM by mixing 20 µL 25 mM Standard with 480 µL dH_2O. Next, dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	Premix + dH_2O	Pyruvate (µM)
1	200 µL + 0 µL	1000
2	120 µL + 80 µL	600
3	60 µL + 140 µL	300
4	0 µL + 200 µL	0

   Transfer 10 µL of each Standard and 10 µL Sample to separate wells in a clear flat-bottom 96 well plate.

2. *Assay*. Prepare enough working reagent (WR) for 4 standards and all samples. For each well combine the following: 95 µL Developer, 1 µL Cosubstrate and 1 µL Dye Reagent. Add 90 µL of WR to each Standard and Sample well. Mix well and incubate protected from light at RT or at desired temperature.

3. *Read* OD_{570nm} at time = 5 min and then again at time = 35 min.

Fluorimetric Procedure

1. *Standards*. Dilute the Standards prepared in Colorimetric Procedure 1:20 in dH_2O.

   Transfer 10 µL Standards and 10 µL Sample into separate wells of a *black* 96-well plate.

2. *Assay*. Add 90 µL Working Reagent (see *Colorimetric Procedure*). Tap plate to mix. Incubate at RT or desired temperature protected from light.

3. Read fluorescence at $\lambda_{ex/em}$ = 530/590 nm at time = 5 min and then again at time = 35 min.

CALCULATION

To determine the slope of the pyruvate standard curve, use the t=35 min measurements. Subtract the blank value (#4) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope by linear regression.

To determine the PK activity in the samples, first subtract the $\mathbf{OD_{5min}}$ from $\mathbf{OD_{35min}}$ or $\mathbf{F_{5min}}$ from $\mathbf{F_{35min}}$ for each sample and the 0 µM Pyruvate Standard (Blank). The PK activity can then be computed as follows:

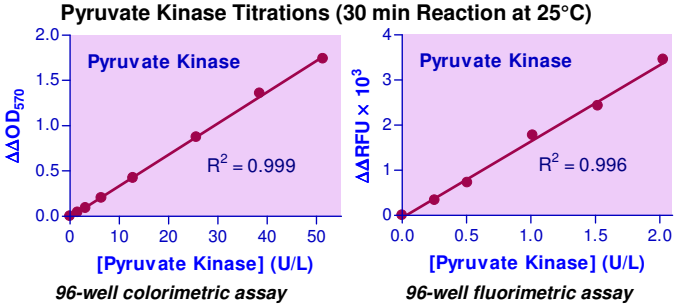
$$[PK] = \frac{\Delta R_{SAMPLE} - \Delta R_{BLANK}}{\text{Slope } (\mu M^{-1}) \times t} \times n \qquad (U/L)$$

where ΔR_{SAMPLE} and ΔR_{BLANK} are the changes over time in the optical density or the fluorescence intensity readings of the Sample and Blank, respectively. ***t*** is the time of reaction (30 min). ***n*** is the sample dilution factor (e.g. *n* = 4 for serum).

Note: if the calculated PK activity is higher than 50 U/L for the colorimetric assay or higher than 2 U/L for the fluorimetric assay, dilute sample in dH_2O and repeat assay. Multiply result by the dilution factor *n*.

Unit definition: 1 unit of PK will generate 1 µmole of pyruvate and 1 µmole ATP from PEP and ADP per minute at 25 °C at pH 7.5.

Pyruvate Kinase Titrations (30 min Reaction at 25°C)



96-well colorimetric assay ***96-well fluorimetric assay***

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, clear or black flat-bottom 96-well plates, plate reader and centrifuge tubes.

LITERATURE

1. Pon NG and Bondar RJ (1967). A direct spectrophotometric assay for pyruvate kinase. Anal. Biochem. 19(2):272-9.

2. Van Rymenant, M. and Robert, J. (1959). Enzymes in cancer. I. Measurement of pyruvate kinase in serum of normal individuals and patients with cancer. Cancer. 12:1087-91.

3. Mosca A, et al (1993). Rapid determination of erythrocyte pyruvate kinase activity. Clin Chem. 39(3):512-6.