Pyruvate Kinase (PK) Assay Kit (Colorimetric/Fluorometric)

LS-K258-100 (100 Tests) • **Store at -20°C**

Introduction

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. LSBio's 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 fluorometric 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.

Components

Component	K258-100
	100 Tests
Developer	12 mL
Cosubstrate	120 µL
Dye Reagent	400 µL
Pyruvate Standard	25 mM

Materials Not Supplied

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

Storage

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

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Assay Procedure

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 OD570nm at time = 5 min and then again at time = 35 min.

Fluorometric 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 λex/em = 530/590 nm at time = 5 min and then again at time = 35 min.

Calculations

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 $\mathbf{\Delta R_{SAMPLE}}$ and $\mathbf{\Delta R_{BLANK}}$ 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 fluorometric 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.

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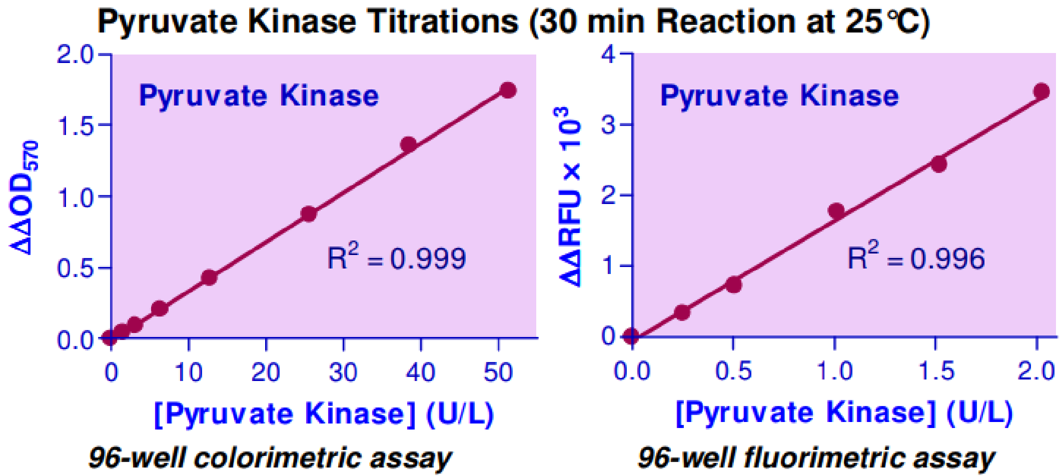
Sample Data



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