

## **Technical Manual**

# **ColorFluor Pyruvate Kinase Activity Assay Kit**

**Catalogue Code: BA0145**

**Pack Size: 100 assays**

**Research Use Only**

## 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.

The Assay Genie ColorFluor Pyruvate Kinase Activity 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  $\lambda_{ex/em} = 530/590\text{nm}$  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)

<b>Developer:</b>	12 mL	<b>Cosubstrate:</b>	120 $\mu\text{L}$
<b>Dye Reagent:</b>	120 mL	<b>Pyruvate Standard:</b>	400 $\mu\text{L}$ 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* ( $2 \times 10^6$ ) can be homogenized in 100  $\mu\text{L}$  PBS. Centrifuge at 14,000 rpm for 5 min. Use clear supernatant for assay.

*Serum* should be diluted at least 4-fold in dH<sub>2</sub>O.

### Colorimetric Procedure

1. **Standards.** First dilute the Pyruvate Standard to 1000  $\mu\text{M}$  by mixing 20  $\mu\text{L}$  25 mM Standard with 480  $\mu\text{L}$  dH<sub>2</sub>O. Next, dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	Premix + dH <sub>2</sub> O	Pyruvate ( $\mu\text{M}$ )
1	200 $\mu\text{L}$ + 0 $\mu\text{L}$	1000
2	120 $\mu\text{L}$ + 80 $\mu\text{L}$	600
3	60 $\mu\text{L}$ + 140 $\mu\text{L}$	300
4	0 $\mu\text{L}$ + 200 $\mu\text{L}$	0

Transfer 10  $\mu\text{L}$  of each Standard and 10  $\mu\text{L}$  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  $\mu\text{L}$  Developer, 1  $\mu\text{L}$  Cosubstrate and 1  $\mu\text{L}$  Dye Reagent. Add 90  $\mu\text{L}$  of WR to each Standard and Sample well. Mix well and incubate protected from light at RT or at desired temperature.
3. **Read**  $\text{OD}_{570\text{nm}}$  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  $\text{dH}_2\text{O}$ .  
Transfer 10  $\mu\text{L}$  Standards and 10  $\mu\text{L}$  Sample into separate wells of a *black* 96-well plate.
2. **Assay.** Add 90  $\mu\text{L}$  Working Reagent (see *Colorimetric Procedure*). Tap plate to mix. Incubate at RT or desired temperature protected from light.
3. Read fluorescence at  $\lambda_{\text{ex/em}} = 530/590 \text{ nm}$  at time = 5 min and then again at time = 35 min.

#### CALCULATION

To determine the slope of the pyruvate standard curve, use the  $t=35 \text{ min}$  measurements. Subtract the blank value (#4) from the standard values and plot the  $\Delta\text{OD}$  or  $\Delta\text{F}$  against standard concentrations. Determine the slope by linear regression.

To determine the PK activity in the samples, first subtract the  $\text{OD}_{5\text{min}}$  from  $\text{OD}_{35\text{min}}$  or  $\text{F}_{5\text{min}}$  from  $\text{F}_{35\text{min}}$  for each sample and the 0  $\mu\text{M}$  Pyruvate Standard (Blank). The PK activity can then be computed as follows:

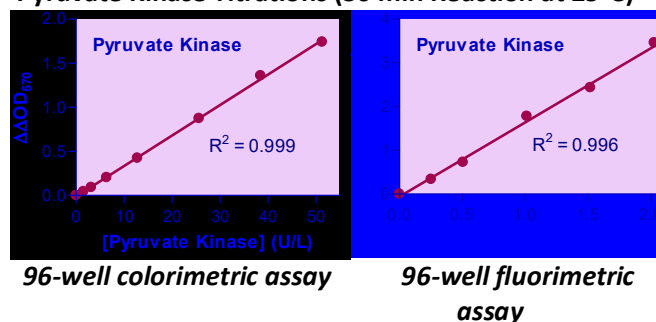
$$[\text{PK}] = \frac{\Delta\text{R}_{\text{SAMPLE}} - \Delta\text{R}_{\text{BLANK}}}{\text{Slope } (\mu\text{M}^{-1}) \times t} \times n \quad (\text{U/L})$$

where  $\text{DR}_{\text{SAMPLE}}$  and  $\text{DR}_{\text{BLANK}}$  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  $\text{dH}_2\text{O}$  and repeat assay. Multiply result by the dilution factor  $n$ .

**Unit definition:** 1 unit of PK will generate 1  $\mu\text{mole}$  of pyruvate and 1  $\mu\text{mole}$  ATP from PEP and ADP per minute at 25  $^\circ\text{C}$  at pH 7.5.

#### Pyruvate Kinase Titrations (30 min Reaction at 25 $^\circ\text{C}$ )



#### 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 Rymentant, 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.

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