# Pyruvate Kinase Assay (PK)

*Cat. No. 8628*
*100 Tests in 96-well plate*

## Introduction

Pyruvate kinase (PK) is an enzyme that catalyzes the final step of glycolysis, the transfer of a phosphate group from phospho(enol) pyruvate to ADP, yielding pyruvate and ATP. Pyruvate kinase can regulate gluconeogenesis and additionally, pyruvate kinase deficiency is the second most common cause of hemolytic anemia. This colorimetric assay is based on pyruvate kinase-catalyzed formation of pyruvate, which can be further oxidized, where the formed hydrogen peroxide is catalyzed by peroxidase and reacts with 4-aminoantipyrine to form a colored product. The intensity of the colored reaction product, which exhibits maximum absorbance at 570 nm, is directly proportional to the activity of pyruvate kinase in the sample.

## Kit Components

| Cat. No. | # of vials | Reagent | Quantity | Storage |
|---|---|---|---|---|
| 8628a | 1 | Assay buffer | 25 mL | 4°C |
| 8628b | 1 | Pyruvate standard | 1 mL | -20°C |
| 8628c | 1 | ADP | 0.6 mL | -20°C |
| 8628d | 1 | Substrate mix | 1.6 mL | -20°C |
| 8628e | 1 | Cofactor mix | 0.4 mL | -20°C |
| 8628f | 1 | Enzyme mix | 0.2 mL | -20°C |

## Product Use

This assay measures PK level in various types of samples, including cells, serum, plasma, and tissue. This product is for research purposes only and is not approved for use in animals, humans, or diagnostic procedures.

## Quality Control

Diluted PK positive control is measured with the PK Assay kit after various reaction times (Figure 2). The linear range of detection is 0.3 to 9.6 U/ml in a 96-well plate assay.

## Shipping

Shipped on dry ice.

**Preparation of positive control**

1. Diluted PK positive control: Add 1 µl of PK positive control into 199 µl of assay buffer (8628a). Prepare diluted PK positive control to a final volume of 10 µL/well in a 96-well flat bottom plate.

**Procedures (96-well plate)**

## A. Preparation of pyruvate standard

1. Add 2 µL of pyruvate standard (8628b) to 48 µL of assay buffer (8628a) to make a 0.05 mL solution of 40 mM pyruvate.
2. Obtain 7 test tubes, add 25 µL of assay buffer (8628a) into each tube and label them #1 through #7.
3. Add 25 µL of the 40mM pyruvate solution into tube #1 and mix well to obtain the 20 mM pyruvate standard.
4. Transfer 25 µL of the 20 mM pyruvate standard from tube #1 to tube #2 and mix well to obtain the 10 mM pyruvate standard.
5. Repeat step 4 for tubes #3-6 to serially dilute the pyruvate standards. Do not add any pyruvate to tube #7, which serves as a blank.
6. Obtain a 96-well test plate, prepare 2 replicates (A, B) of each pyruvate standard by aliquoting 10 µL/well of each pyruvate standard into duplicate wells of the 96-well test plate, following plate format below:

| | #1 | #2 | #3 | #4 | #5 | #6 | #7 |
|---|---|---|---|---|---|---|---|
| **A** | 20 mM | 10 mM | 5 mM | 2.5 mM | 1.25 mM | 0.625 mM | Blank |
| **B** | 20 mM | 10 mM | 5 mM | 2.5 mM | 1.25 mM | 0.625 mM | Blank |

## B. Preparation of test samples

1. Cells or Tissues can be homogenized in 4 volumes of the assay buffer (8628a). Centrifuge the samples at 13,000 ×g for 10 minutes to remove insoluble material. The soluble fraction may be assayed directly.
2. Samples should be serial diluted to make sure the readings are within the range of the standard curve. Prepare test samples to a final volume of 10 µL/well in the 96-well flat bottom plate.

## C. Preparation of working reagents and measurements

1. Prepare the appropriate volume of PK assay working reagent based on the number of samples to be measured. For each well of pyruvate standard, diluted PK positive control and blank, prepare the working reagent by mixing 72 µL of assay buffer (8628a), 6 µL of ADP (8628c), 16 µL of substrate mix (8628d), 4 µL of cofactor mix (8628e), and 2 µL of enzyme mix (8628f).
2. Add 90 µL of the working reagent mix into each well of the 96-well plate containing pyruvate standard, diluted PK positive control, sample, and blank. Incubate the reaction for 20 minutes at room temperature in the dark.
3. Measure the absorbance readings at 570 nm with an ELISA plate reader at 0 minutes and 20 minutes.

## D. Calculations

1. Subtract the $OD_{570nm}$ value of the blank from the $OD_{570nm}$ values of all the standards and samples to obtain the $\Delta OD_{570nm}$ values.
2. Based on the calibrated $\Delta OD_{570nm}$ of the pyruvate standard, make a standard curve by plotting $\Delta OD_{570nm}$ as a function of pyruvate concentration (See Figure 1 for a typical standard curve). Determine the slope and $R^2$ value of the resulting trend line.

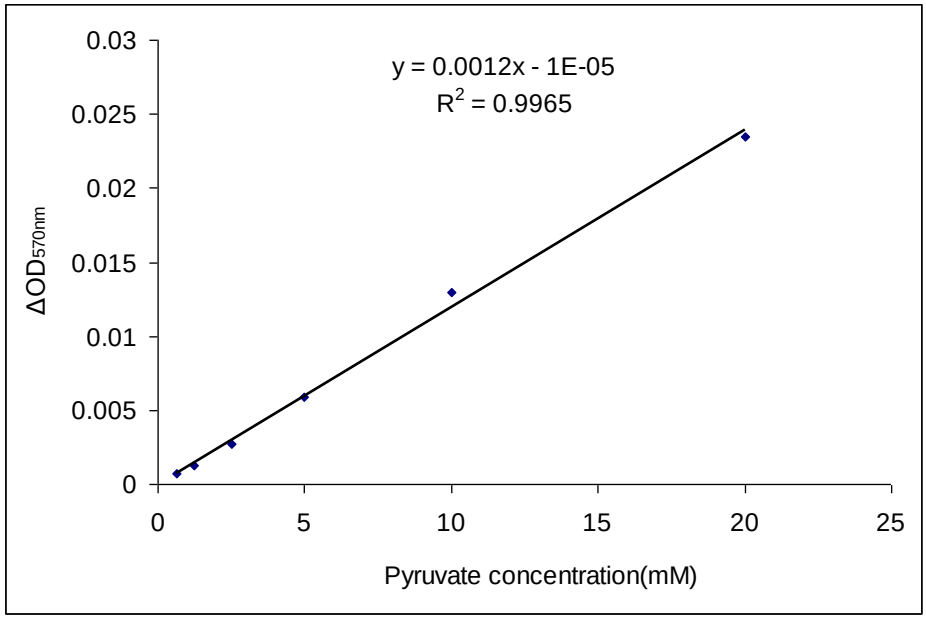


Figure 1. A typical pyruvate standard curve measured by ScienCell™ Pyruvate Kinase Assay kit.

3. Calculate the PK activity of the samples and diluted PK positive control using the following formula:

$$[\text{Pyruvate Kinase}] = \frac{T_{20} - T_0}{\text{Slope X } 20} \text{ x sample dilution}$$

**Note:** $T_{20}$ and $T_0$ are absorbance readings of the sample and positive control at 20 minutes and 0 minutes, respectively. 20 is the enzyme reaction time.

**Unit definition:** One unit catalyzes the formation of 1.0 µmol of pyruvate per minute at 25ºC, pH 8.0.

4. Obtain the change in absorbance, $T_{20}$ and $T_0$, based on the curve of the diluted PK positive control, as shown in Figure 2.

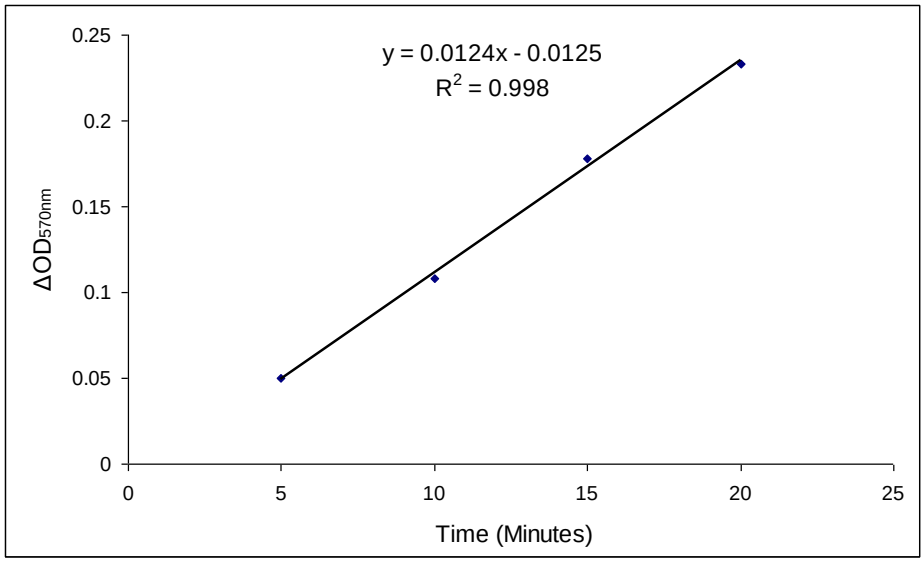


Figure 2. The change in absorbance, $\Delta OD_{570nm}$, of diluted PK positive control during the indicated time at 570nm.

5. Use the formula below to calculate the activity of PK positive control:

$$[\text{Pyruvate kinase}] = \frac{0.233}{0.0012 \text{ X } 20} \text{ x } 200 = 1.941 \text{ (U/ml)}$$