



Pyruvate Kinase Assay (PK)

Catalog #8258

100 Tests in 96-well plate

Product Description

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

Additional Materials Required (Not provided):

Pyruvate kinase positive control

Microplate reader

96-well plates with clear flat-bottom

Tubes required for preparation of reagents

Product Use

PK kit evaluates the pyruvate kinase activity *in vitro* and is for research use only. It is not approved for human or animal use or for applications in clinical or *in vitro* 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

Kit components are shipped on dry ice. Upon receipt store 8628b, 8628c, 8628d, 8628e and 8628f at -20°C and 8628a at 4°C.

Preparation of positive control (Optional)

1. Prepare dilution of PK positive control in assay buffer (8628a) 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 $\times g$ for 10 minutes to remove insoluble material. The soluble fraction may be assayed directly.
2. Samples should be serially 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 ΔOD_{570nm} values.
2. Based on the calibrated ΔOD_{570nm} of the pyruvate standard, make a standard curve by plotting ΔOD_{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.

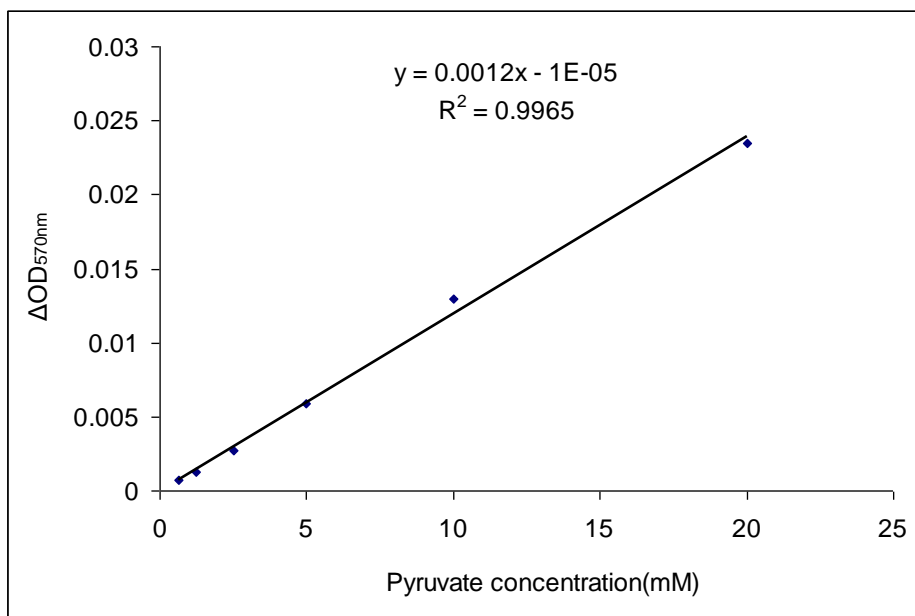


Figure 1. A typical pyruvate standard curve measured by ScienCell™ Pyruvate Kinase Assay kit.

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

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

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 25°C, 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.

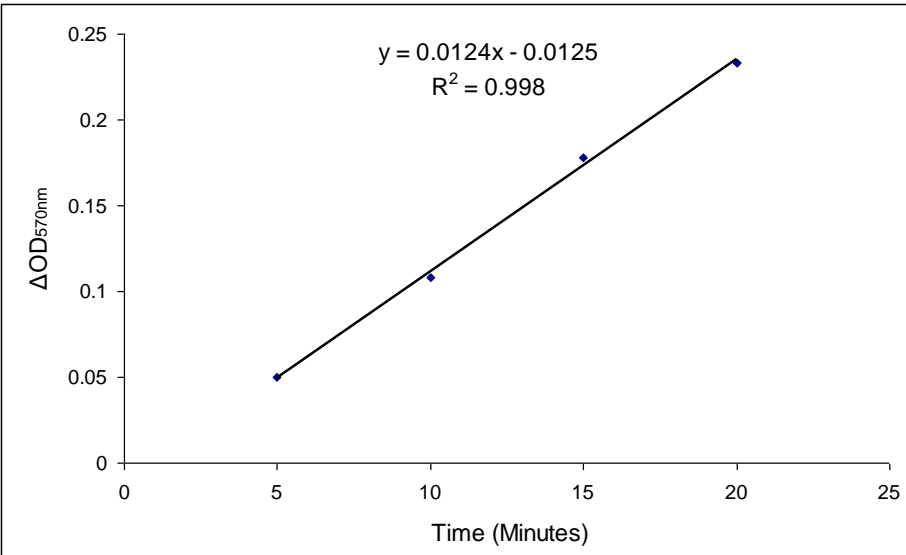


Figure 2. The change in absorbance, ΔOD_{570nm} , of diluted PK positive control during the indicated time at 570nm.

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

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