Pyruvate Assay (PYR)

Cat. No. 8388
100 Tests in 96-well plate

Introduction

Pyruvate is a central molecule in cellular metabolic pathways. Pyruvate can be converted to carbohydrates via gluconeogenesis, to fatty acids or energy through acetyl-CoA, to the amino acid alanine, and to ethanol. Abnormal levels of pyruvate have been linked to liver diseases and metabolic disorders. This colorimetric assay is based on pyruvate oxidase catalyzed oxidation of pyruvate, in which the formed hydrogen peroxide is catalyzed by peroxidase and reacts with 4-aminoantipyrine to form the product dye. The color intensity of the reaction product, which exhibits maximum absorbance at 570 nm, is directly proportional to pyruvate concentration in the sample.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8388a	1	Assay buffer	10 mL	4°C
8388b	1	Pyruvate standard	1 mL	-20°C
8388c	1	Substrate mix	1.6 mL	-20°C
8388d	1	Cofactor mix	0.4 mL	-20°C
8388e	1	Enzyme mix	0.2 mL	-20°C

Product Use

This assay measures pyruvate level of samples from cells, serum, plasma, and tissue. It is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Quality Control

Data from Pyruvate Assay of pyruvate solutions with concentrations ranging from 0.05 to 20 mM show a linear relationship between OD_{570nm} and pyruvate concentration (Figure 1).

Shipping

Shipped on dry ice.

Procedure (96-well plate)

A. Preparation of pyruvate standard

1. Add 2 µL of pyruvate standard (8388b) to 48 µL of assay buffer (8388a) to make a 0.05 mL solution of 40 mM pyruvate.
2. Obtain 7 test tubes, add 25 µL of assay buffer (8388a) into each tube and label them #1 through #7.
3. Add 25 µL of the 40mM into tube #1 and mix well to get 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 get the 20 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 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, according to the following plate format:

	#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 (8388a). 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 standard curve range. Prepare test samples to a final volume of 10 µL/well on the 96-well flat bottom plate.

Recommendation:

Endogenous enzymes may degrade pyruvate quickly. Samples (such as cell or tissue lysate) should be deproteinized with a 10k Da MW spin filter (Millipore UFC501008) or 0.5M metaphosphoric acid (Sigma 239275) and kept at -80°C for storage.

C. Working reagent preparation and measurements

1. For each well of reaction, prepare working reagent by mixing 68 µL assay buffer (8388a), 16 µL substrate mix (8388c), 4 µL cofactor mix (8388d), and 2 µL enzyme mix (8388e).
2. Add 90 µL of working reagent mix into each well of the 96-well plate containing pyruvate standard, samples, and blank. Incubate for 60 minutes at room temperature protected from light.
3. Read the absorbance at 570 nm with an ELISA plate reader.

D. Calculations

1. Subtract the OD_{570nm} value of the blank from the OD_{570nm} values obtained with all other standard and samples to get ΔOD_{570nm} value.
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 equation and R^2 value of the trend line.
3. Because the equation of the trend line of the standard curve is $y = Ax + B$, calculate the pyruvate concentration of test samples as follows:

$$[\text{pyruvate}] = \frac{\Delta\ OD_{570nm} - B}{A}$$

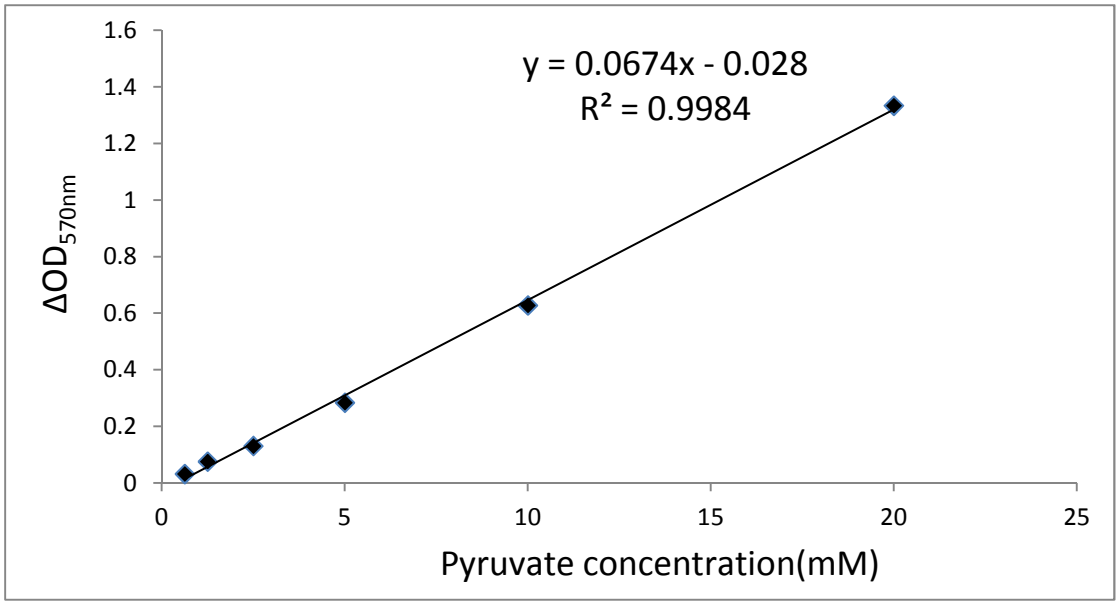

Figure1. A typical pyruvate standard curve measured by ScienCell™ Pyruvate Assay kit