Amplite™ Colorimetric Pyruvate Assay Kit

Catalog number: 13821
Unit size: 200 Tests

Component	Storage	Amount
Component A: Quest Fluor™ Pyruvate Sensor	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B1: Enzyme Mix 1	Freeze (< -15 °C), Minimize light exposure	2 bottles (lyophilized powder)
Component B2: Enzyme Mix 2	Freeze (< -15 °C), Minimize light exposure	2 vials (lyophilized powder)
Component C: Assay Buffer	Freeze (< -15 °C)	1 bottle (10 mL)
Component D: Pyruvate Standard	Freeze (< -15 °C), Minimize light exposure	1 vial (100 mM, 100 µL)
Component E: DMSO	Freeze (< -15 °C)	1 vial (100 µL)

OVERVIEW

Pyruvate is an important chemical compound in intracellular metabolic pathways. It is derived from metabolism of glucose known as glycolysis. One molecule of glucose breaks down into two molecules of pyruvate, which supplies living cells energy through one of two ways. When oxygen is present (aerobic respiration), pyruvate is converted into acetyl-CoA by pyruvate dehydrogenase which enters citric acid cycles (also known as the Krebs cycle) to generate ATP. When there is insufficient oxygen is available, the pyruvate is broken down anaerobically, creating lactate in animals and ethanol in plants and microorganisms. Abnormal levels of pyruvate, or concentration ratio of lactate-to-pyruvate may be linked to liver disease or metabolic disorders and it is a diagnostic measurement in patient's clinical and other laboratory studies. AAT Bioquest's Amplite™ Fluorimetric Pyruvate Assay Kit offers a sensitive fluorescent assay for quantifying pyruvate in biological samples. It utilizes an enzyme coupled reaction that releases hydrogen peroxide, which can be detected by pyruvate sensor in a fluorescence microplate reader.

AT A GLANCE

Protocol Summary

1. Prepare test samples along with serially diluted pyruvate standards (50 µL)
2. Add equal volume of working solution (50 µL)
3. Incubate at room temperature for 30 minutes to 1 hour
4. Monitor absorbance intensity at 575 nm

Important Thaw kit components at room temperature before use.

KEY PARAMETERS

Absorbance microplate reader

Absorbance	575 nm
Recommended plate	Clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

Quest Fluor™ Pyruvate Sensor stock solution (200X)
Add 50 µL of DMSO (Component E) into Quest Fluor™ Pyruvate Sensor (Component A) to make 200X Quest Fluor™ Pyruvate Sensor stock solution.

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/13821

Pyruvate standard

Add 2 µL of 100 mM Pyruvate (Component D) into 998 µL of PBS (pH 7.0) to have 200 µM Pyruvate standard solution (PS7). And then perform 1:2 serial dilutions to get remaining serially diluted pyruvate standards (PS6 - PS1).

PREPARATION OF WORKING SOLUTION

Add 5 mL Assay Buffer (Component C) into one Enzyme Mix1 bottle (Component B1) and mix well. Add 100 µL of ddH_2O into one Enzyme Mix2 vial (Component B2) and mix well. Transfer entire vial of Enzyme Mix2 (100 µL) and 25 uL of 200X pyruvate sensor stock solution into the Enzyme Mix1 bottle and mix well.

Note The working solution is not stable. Use promptly and avoid direct exposure to light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of pyruvate standards and test samples in a white clear 96-well microplate. PS=Pyruvate Standard (PS1 - PS7, 3.125 to 200 µM), BL=Blank Control (PBS), TS=Test Sample.

BL	BL	TS	TS
PS1	PS1	...	...
PS2	PS2	...	...
PS3	PS3		
PS4	PS4		
PS5	PS5		
PS6	PS6		
PS7	PS7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
PS1 - PS7	50 µL	Serial Dilutions (3.125 to 200 µM)
BL	50 µL	PBS
TS	50 µL	test sample

1. Prepare pyruvate standards (PS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of working solution to each well of pyruvate standard, blank control, and test samples to make the total pyruvate assay volume of 100 µL/well. For a 384-well plate, add 25 µL of working solution into each well instead, for a total volume of 50 µL/well.

   Note Run the pyruvate assay at pH 6.5 to 7.0.

3. Incubate the reaction mixture at room temperature for 30 minutes to 1 hour.

4. Monitor the absorbance increase with an absorbance microplate reader at 575 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Absorbance) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain

the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Pyruvate samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

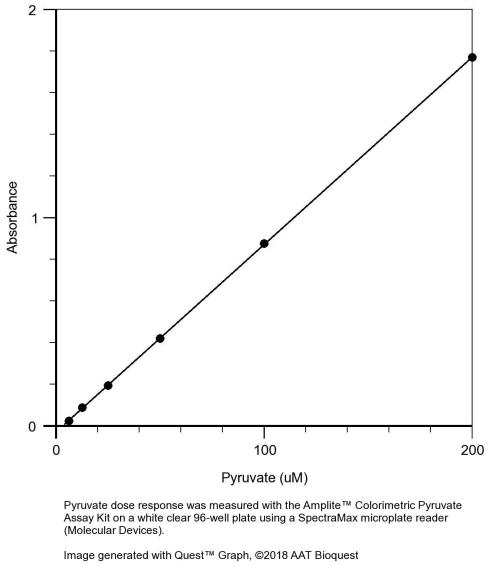

Figure 1. Pyruvate dose response was measured with the Amplite™ Colorimetric Pyruvate Assay Kit on a white clear 96-well plate using a SpectraMax microplate reader (Molecular Devices).

DISCLAIMER

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