Amplite™ Fluorimetric Pyruvate Assay Kit

Catalog number: 13820
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	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™ Colorimetric Pyruvate Assay Kit offers a sensitive colorimetric assay for quantifying pyruvate in the samples. It utilizes an enzyme coupled reaction that releases hydrogen peroxide, which can be detected by pyruvate sensor by an absorbance microplate reader at 575 nm.

AT A GLANCE

Protocol summary

1. Prepare test samples (50 µL) along with serially diluted pyruvate standards (50 µL)
2. Add equal volume of pyruvate working solution (50 µL)
3. Incubate at room temperature for 30 minutes to 1 hour
4. Monitor fluorescence intensity at Ex/Em = 540/590 nm

Important To achieve the best results, it's strongly recommended to use the black plates. Thaw kit components at room temperature before use.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Solid black

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.

1. Quest Fluor™ Pyruvate sensor stock solution (200X):
Add 55 µL of DMSO (Component E) into Quest Fluor™ Pyruvate Sensor (Component A) to make 200X Quest Fluor™ Pyruvate sensor stock solution.

2. Pyruvate standard solution (1 mM):
Add 10 µL of 100 mM Pyruvate (Component D) into 990 µL of PBS (pH 7) to get 1 mM pyruvate solution.

PREPARATION OF STANDARD SOLUTION

Pyruvate standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/13820

Take 100 µL of 1 mM pyruvate standard solution into 900 µL PBS to make 100 µM pyruvate solution (PS7). And then perform 1:3 serial dilutions to get serially diluted pyruvate standards (PS6 - PS1).

PREPARATION OF WORKING SOLUTION

1. Add 5 mL Assay Buffer (Component C) into one Enzyme Mix 1 bottle (Component B1); mix well.

2. Add 100 µL of ddH_2O into one Enzyme Mix 2 vial (Component B2); mix well.

3. Transfer entire vial (100 µL) of Enzyme Mix 2 and 25 uL of 200X Quest Fluor™ Pyruvate Sensor stock solution into the Enzyme Mix 1 bottle. Mix well.

Note The pyruvate working solution is not stable and should be used promptly. Avoid light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of pyruvate standards and test samples in a solid black 96-well microplate. PS = pyruvate standard (PS1 - PS7, 0.1 to 100 µM); BL = blank control; 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 dilution (0.1 to 100 µM)
BL	50 µL	PBS
TS	50 µL	sample

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1. Prepare pyruate standards (PS), blank controls (BL), and test samples (TS) into a solid black 96-well microplate according to the layout provided in Table 1 and Table 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of pyruvate working solution into 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. Run the pyruate assay at a pH of 6.5 to 7.0.

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

4. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 540/590 nm (cut off: 570 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) 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

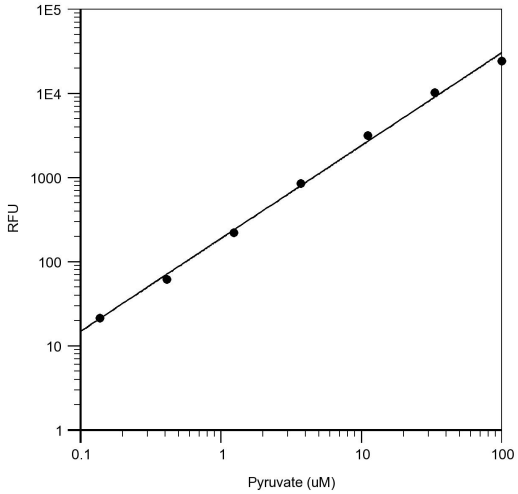

Figure 1. Pyruvate dose response was measured with the Amplite™ Fluorimetric Pyruvate Assay Kit on a solid black 96-well plate using a Gemini microplate reader (Molecular Devices).

DISCLAIMER

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