Amplite™ Colorimetric Pyruvate Assay Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
#Cat: 13821 (200 Assays)	Keep in freezer and protect from light	Absorbance microplate readers

Introduction

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.

Kit Components

Components	**Amount**
Component A: Quest Fluor™ Pyruvate Sensor	1 vial
Component B1: Enzyme Mix1	2 bottles (lyophilized powder)
Component B2: Enzyme Mix2	2 vials (lyophilized powder)
Component C: Assay Buffer	1 bottle (10 mL)
Component D: Pyruvate Standard	100 mM (100 µL)
Component E: DMSO	1 vial (100 µL)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare test samples (50 µL) along with serially diluted pyruvate standards (50 µL) → Add equal volume of Assay Mixture (50 µL) → Incubate at room temperature for 30 minutes to 1 hour → Monitor absorbance intensity at 575 nm

1. Prepare Pyruvate Assay Mixture:

1.1 Thaw kit components at room temperature before use.

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

1.3 Make Assay Mixture:

1.3.1 Add 5mL Assay Buffer (Component C) into one Enzyme Mix1 bottle (Component B1) mix well.

1.3.2 Add100 µL of ddH_2O into one Enzyme Mix2 vial (Component B2) mix well.

1.3.3 Transfer entire vial (100 µL) of Enzyme Mix2 (from Step 1.3.2) and 25 uL of 200X pyruvate sensor stock solution (from Step 1.2) into the Enzyme Mix1 bottle (from Step 1.3.1) and mix well.

Note1: The assay mixture is not stable, use it promptly, and avoid direct exposure to thelight.

Note2: Store unused 200 X Quest Fluor™ Pyruvate sensor stock solution at -20°C, avoid light and repeat freeze-thaw cycles.

2. Prepare serially diluted pyruvate standards and test samples:

2.1 Prepare pyruvate standard: Add 2 µL of 100 mM Pyruvate (Component D) into 998 µL of PBS (pH 7.0) to have 200 µM pyruvate solution. And then perform 1:2 serial dilutions to get 100, 50, 25, 12.5, 6.25, and 3.125 µM serially diluted pyruvate standards.

2.2 Add pyruvate containing samples and serially diluted pyruvate standards into a white clear 96-well microplate according to Tables 1.

Table 1 Layout of pyruvate standards and test samples in a white clear 96-well microplate

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

Note 1: PS= Pyruvate Standard, BL=Blank Control (PBS), TS=Test Sample.
Note 2: Add the serial dilutions of pyruvate standard from 0.1 µM to 100 µM into wells from PS1 to PS7.

3. Run pyruvate assay:

3.1 Add 50 µL of Assay Mixture (from Step 1.3.3) into each well of pyruvate standard, blank control, and test samples (see Step 2.2) to make the total pyruvate assay volume of 100 µL/well.
Note 1: For a 384-well plate, add 25 µL of sample, 25 µL of Assay mixture (from Step 1.3) into each well.
Note 2: Run the pyruvate assay at pH 6.5 to 7.0.
3.2 Incubate the reaction mixture at room temperature for 30 minutes to 1 hour.
3.3 Monitor the absorbance increase with an absorbance microplate reader at 575 nm.

Data Analysis

The absorbance reading in blank wells (with assay buffer only) is used as a control, and is subtracted from the values of those wells with the pyruvate standards and test samples. A pyruvate standard curve is shown in Figure 1. Calculate the pyruvate concentrations of the samples according to the pyruvate standard curve.

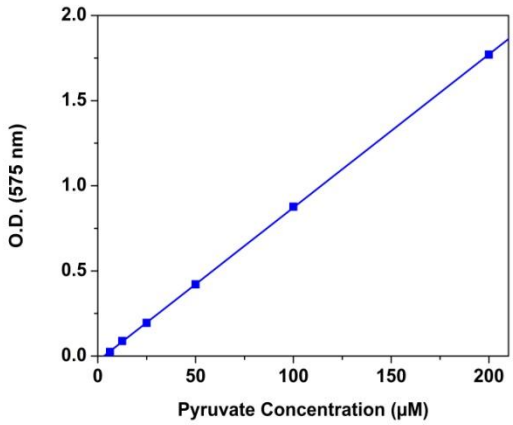

Figure1. Pyruvate dose response was measured with the Amplite™ Colorimetric Pyruvate Assay Kit on a white clear 96-well plate using a SpectraMax microplate reader (Molecular Devices). As low as 6 µM of pyruvate can be detected with 30min incubation (*Note: The absorbance background increases with time, thus it is important to subtract the absorbance intensity value of the blank wells for each data point).*

References

1. Olenchock, Benjamin A., and Matthew G. Vander Heiden. "Pyruvate as a Pivot Point for Oncogene-Induced Senescence." *Cell* 153.7 (2013): 1429-1430.
2. Sanchez, Jose J., et al. "Neuromonitoring with Microdialysis in Severe Traumatic Brain Injury Patients." *Brain Edema XV*. Springer Vienna, 2013. 223-227.
3. Singh, Sunil Kumar, Shailendra K. Singh, and Ajay Singh. "Toxicological and biochemical alterations of apigenin extracted from seed of Thevetia peruviana, a medicinal plant." *Journal of Biology and Earth Sciences* 3.1 (2013): B110-B119.