Product Information

Pyruvate Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2119-100 100 assays	Cap Color	Part Number
Pyruvate Assay Buffer	25 ml	WM	K2119-C-1
Pyruvate Probe (in DMSO)	200 µl	Red	K2119-C-2
Pyruvate Enzyme Mix	Lyophilized	Green	K2119-C-3
Pyruvate Standard (100 nmol/µl)	100 µl	Yellow	K2119-C-4

II. Introduction:

Pyruvate is an important intermediate in several metabolic pathways. Pyruvate is produced from glucose via glycolysis. Pyruvate can be converted to fatty acids via acetyl CoA or to carbohydrates via gluconeogenesis. Pyruvate is also used to stimulate metabolism which then leads to loss of body weight. High levels of pyruvate are associated with genetic disorders and liver disease.

The Pyruvate Colorimetric/Fluorometric Assay Kit provides a simple and convenient way for detection of pyruvate in various biological samples such as culture and fermentation media, blood and cells based on colorimetric and fluorometric method. In the assay, pyruvate is oxidized by pyruvate oxidase via enzyme reactions to yield fluorescence (at Ex/Em = 535/587 nm) and color (at λ = 570 nm). The fluorescence or color intensity is proportional to pyruvate content. The assay provides a direct and automation-ready procedure for accurately measuring pyruvate concentration. The kit can detect 1 µM to 10 mM pyruvate.

III. Application:

Measurement of pyruvate in various tissues/cells/serum/saliva etc.

Analysis of metabolism in various cells.

IV. Sample Type:

Animal tissues.

Cell culture: adherent or suspension cells.

Serum, saliva.

V. User Supplied Reagents and Equipment:

96-well plate with flat bottom.

Multi-well spectrophotometer.

VI. Storage and Handling:

Store kit at -20℃, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

Pyruvate Assay Buffer: Warm to room temperature before use. Store at 4℃ or -20℃.

Pyruvate Probe: Briefly warm at 37℃ for 1-2 min. to completely melt the DMSO solution. Mix well, store at –20℃, protected from light and moisture. Use within two months.

Pyruvate Enzyme Mix: Dissolve with 220 µl Pyruvate Assay Buffer. Pipette up and down to completely dissolve. Store at –20℃. Use within two months.

VIII. Pyruvate Assay Protocol:

1. Sample Preparation: Serum can be directly added into sample wells (serum contains ~ 50 - 100 pmol/µl pyruvate). Centrifuge saliva at 10,000 x g for 10 min. at 4℃. Add 10 - 20 µl into 96-well plate. Adjust volume to 50 µl/well with Pyruvate Assay Buffer. Tissues or cells can be extracted with 4 volume of the Pyruvate Assay Buffer. Centrifuge (10000 x g; 10 min.; 4℃) to remove insoluble material. Collect supernatant. Add 2 - 50 µl samples into 96-well plate. Adjust the volume to 50 µl/well with Pyruvate Assay Buffer. Samples should be stored at -80℃ if assayed later.

Notes:

a. For unknown samples, we suggest performing a pilot experiment & testing different sample dilutions to ensure the readings are within the Standard Curve range.

b. Due to the presence of LDH in serum, care must be taken during sample processing to prevent the conversion of pyruvate to lactate.

c. Samples can be deproteinized by 10K Spin Column to remove proteins that consume pyruvate.

d. For samples having background, prepare parallel well(s) containing same amount of sample as in the test well.

e. Endogenous compounds may interfere with the reaction. To ensure accurate determination of Pyruvate in the test samples, we recommend spiking samples with a known amount of Standard (6 nmol).

2. Standard Curve Preparation: For colorimetric assay: dilute the Pyruvate Standard to 1 nmol/µl by adding 10 µl of the Standard to 990 µl of Pyruvate Assay Buffer, mix well. For fluorometric assay: Dilute the Pyruvate Standard to 1 nmol/µl as for the colorimetric assay. Then further dilute the Standard to 0.1 nmol/µl by adding 10 µl of 1 nmol/µl Standard into 90 µl of Pyruvate Assay Buffer. Mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells in 96-well plate. Adjust the volume to 50 µl/well with Pyruvate Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Pyruvate Standard for the colorimetric assay (0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well for the fluorometric assay).

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix containing the following components. Mix well before use:

	Reaction Mix	Background Control Mix
Pyruvate Assay Buffer	46 µl	48 µl
Pyruvate Probe	2 µl	2 µl
Enzyme Mix	2 µl	---

Add 50 µl of the Reaction Mix to each well containing the Pyruvate Standard & test samples, mix well.

For samples having background, add 50 µl of Background Control Mix to sample background control well(s).

For fluorometric assay use 0.4 µl Pyruvate Probe and 47.6 µl Pyruvate assay buffer to reduce background.

4. Measurement: Incubate the reaction for 30 min. at room temperature. Protect from light. Measure absorbance (OD 570 nm) or fluorescence (Ex/Em = 535/590 nm) in a microplate reader.

5. Calculation: Subtract 0 Standard reading from all readings. If sample background control reading is significant then subtract the sample background control reading from sample readings. Plot the Pyruvate Standard Curve. For unspiked samples, apply the corrected absorbance or fluorescence to the Pyruvate Standard Curve to get B nmol of Pyruvate in the sample well.

Sample Pyruvate concentration (C) = B/V X D (nmol/µl or mM)

Where: B is the amount of pyruvate in the sample well (nmol).

V is the sample volume added into the reaction well (µl).

D is the sample dilution factor.

Note: For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

For spiked samples, Pyruvate amount in sample well (B) =(OD $_{\text{sample (corrected)}}$/ (OD $_{\text{sample + Pyruvate Std(corrected)}}$ - OD $_{\text{sample (corrected)}}$))* Pyruvate Spike (pmol).

Pyruvate molecular weight: 88.08.

Pyruvate concentration in your samples can be expressed as nmol/ml, or mg/ml, or mg/dL or mM (mmol/liter). 1 mM = 8.81 mg/dl.

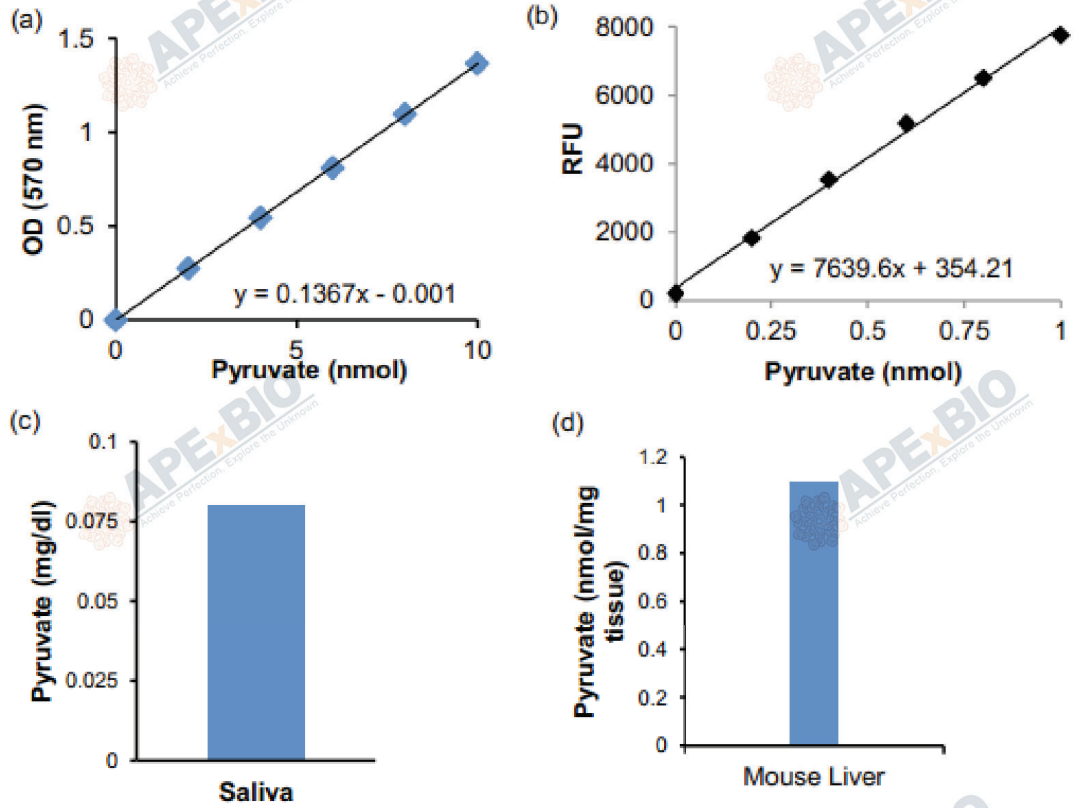

Figure: Pyruvate Standard Curve: colorimetric (a), fluorometric (b). Quantitation of pyruvate in human saliva (c) and mouse liver lysate (d). Saliva sample was centrifuged at 10,000 x g for 10 min. at 4°C. Supernatant (10 µl) was spiked with known amounts of pyruvate Standard (6 nmol) and assayed according to kit protocol. Mouse liver homogenate was centrifuged and supernatant was collected. Supernatant was deproteinized using 10 kDa spin column and filtrate was diluted 5 fold. 10 µl of this diluted filtrate was spiked with known amount of pyruvate Standard (6 nmol) and assayed according to kit protocol.

Frequently Asked Questions:

1. What is the process to use this kit with adherent cells?

If cells are collected by Trypsinization,it is essential to neutralize the Trypsin with medium. Then all media must be removed and cells washed with PBS. For measuring pyruvate start with 1 - 2 x 10^6 cells, suspend the cell pellet in 3 - 4 volumes of the Pyruvate assay buffer on ice, homogenize using a Dounce homogenizer (10 - 50 passes) on ice, until efficient lysis is confirmed, by viewing the cells under the microscope. Spin down the sample and collect the supernatant. Load the supernatant unto a 10 kda spin column for deproteinzation. Use the eluate for your subsequent assays. Appropriate dilutions of the sample must be tested in order ensure the readings will fall within the linear range of the standard curve.

2. The OD values are very low. What could be the problem?

The most common reason for low OD values is that the Assay buffer was cold, which led to a slow reaction.

3. Is it possible that the pyruvate in the sample is degraded with time?

Yes, pyruvate is lost fairly rapidly and can get used up by cellular enzymes. It is essential to deproteinize the samples as soon as possible.

4. Should the samples be deproteinized and stored?

Yes, it is recommended that cell/tissue lysates or media is deproteinized and then stored at -80℃ if needed.

5. How much pyruvate is there is serum versus whole blood?

Typically, normal human serum has a pyruvate concentration in the range of 60 - 150 μM and normal human blood has a pyruvate concentration in the range of 35 - 100 μM.

6. What kind of interfering substances can affect the assay results? Undiluted deproteinized samples show values above the expected/reported range.

Compounds such as lactate and α-keto acids in the samples are known to have potential to interfere with the measurement of pyruvate, particularly, 2-Oxobutyrate and oxaloacetate. This could explain why the undiluted deproteinized samples suffer from overestimation.

7. Why are my standard curve values lower than those shown on the datasheet?

There are multiple factors which influence the signals like the incubation times, room temperature, handling etc. In general, to increase the value of the standards, you can increase the incubation time. As long as the standard curve is linear, it should be fine to use, since all of your samples will also be measured under the same conditions on this curve.

8. Is it possible to use a different wavelength than recommended for the final analysis?

It is always recommended to use the exact recommended wavelength for the most efficient results. However, most plate readers have flexibility in their band width of detection in increments of +/- 10 nm. Depending on this flexibility range, you can deviate from the recommended wavelengths within limits.

9. Can this kit be used to measure pyruvate in yeast or bacterial cells?

This assay protocol has been optimized for samples of mammalian origin but theoretically this assay should work with samples from multiple origins including bacteria and yeast. Special lysis reagents might be required and the sample amount might need optimization for best results.

10. Will the phenol red in the media affect the assay readout?

Very low amounts of media are used for each sample. This will generate a very low background at the best. Please use only media as a background control and subtract this reading from all sample readings to accommodate for the phenol red.

11. Why is it essential to use less probe for the fluorometric assay?

The fluorometric assay is at least 10 times more sensitive and hence too much probe can saturate the detector of the fluorometer. Therefore 5 times less probe is used..

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If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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