MA-0129

Pyruvate Assay

(100 wells, Colorimetric, OD 570 nm or Fluorometric, Ex/Em = 535/580 nm, Store at -20°C)

Background Information:

Pyruvate is a crucial molecule in cellular metabolism. It is the end product of glycolysis, the initial step in both aerobic and anaerobic respiration. In aerobic conditions, pyruvate enters mitochondria where it undergoes further oxidation to produce energy-rich molecules like ATP through the Krebs cycle and oxidative phosphorylation. Alternatively, under anaerobic conditions, pyruvate can be converted into lactate, regenerating the necessary cofactor NAD+ for glycolysis to continue. Pyruvate also serves as a key precursor for various biosynthetic pathways, including the synthesis of amino acids and fatty acids. Its central role in energy production and biosynthesis makes pyruvate a fundamental molecule in cellular processes. The AkrivisBio Pyruvate Assay is a simple sensitive means of quantitating pyruvate in the range from below 1 µM – 10 mM from a variety of biological sample types.

Assay Principle:

1 – Pyruvate in the sample is oxidized by pyruvate oxidase with the formation of hydrogen peroxide.
2 – The peroxide is utilized by peroxidase to convert ADHP to resorufin giving intense color (570 nm) and fluorescence (excitation 535nm; emission 580 nm).

Kit Contents:

Assay Buffer	25 ml	WM	MA-0129-A
ADHP Solution	200 µl	Red	MA-0129-B
Pyruvate Oxidase/Peroxidase	Lyophil.	Green	MA-0129-C
Pyruvate Standard	100 µl	Yellow	MA-0129-D

Storage and Handling:

Store all components at -20°C. Bring to room temperature before use. Centrifuge vials for a few seconds, prior to opening.
Assay Buffer: Warm to room temperature before use. Store at 4°C.
ADHP Solution: Warm to room temperature to melt DMSO solution. Mix well, store at –20°C.
Pyruvate Oxidase/Peroxidase: Add 220 µl Assay Buffer to dissolve. Store at –20°C

Assay Protocol:

1. **Standard Curve:**
a. Absorbance based assay (~100 µM – 10 mM in samples): Dilute pyruvate standard to 0.4 mM by transferring 10 µl of the Standard to 990 µl of Assay Buffer.
b. Fluorescence based assay (~0.2 µM–100 µM): Dilute pyruvate standard to a final concentration of 0.04 mM by adding 10 µl of the standard to 990 µl of assay buffer as in the absorbance-based assay, then dilute further by transferring 10 µl to 90 µl of Assay Buffer.
c. Mix well. Add 0 – 5 – 10 – 15 – 20 – 25 µl into a series of wells in a 96-well plate. Adjust all well volumes to 50 µl Assay Buffer giving 0, 2, 4, 6, 8, 10 nmol/well of the Pyruvate Standard for the colorimetric assay. (0, 200, 400, 600, 800, 1000 pmol/well for the fluorescence assay).
2. **Sample Preparation:** Homogenize tissue (10 mg) or cells (10^6) with 50 µl of assay buffer. Centrifuge at 16,000 X g, 5 minutes, then add 5-50 µl of the clear supernatant to wells in a 96 well plate. Centrifuge saliva at 16,000 X g for 5 min. at 4°C, then add 10 µl to wells. Serum (10 µl) can be added directly to sample wells. Adjust all well volumes to 50 µl with Assay Buffer.

Notes:
a. LDH activity in serum can convert pyruvate to lactate. Serum samples should be kept at -80°C. Use immediately upon thawing.
b. Deproteinize samples using a 10 kDa Spin Column to remove enzymes that can consume pyruvate.
c. Some samples give a high background. So, run samples in duplicate with the paired well used to determine background.
d. In rare cases, endogenous compounds interfere with the enzyme reaction. Use paired samples with an internal standard added to one of the test samples (2-5 nmol for absorbance, 100 pmol for fluorescence) to measure pyruvate accurately (procedure explained below).

3. **Initiate Reaction:** Prepare sufficient Reaction Mix for the total number of wells to be run. Each well requires 50 µl of Reaction Mix containing:
Reaction Mix:

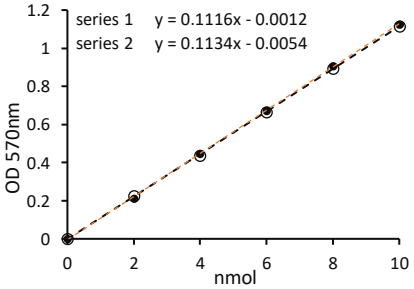
	Sample/Standard Well Mix	Background Control Mix
Assay Buffer	46 µl (47.6 µl using fluorescence)	48 µl (49.6 µl using fluorescence)
ADHP Solution	2 µl (0.4 µl using fluorescence)	2 µl (0.4 µl using fluorescence)
Pyruvate Oxidase/Peroxidase	2 µl	----

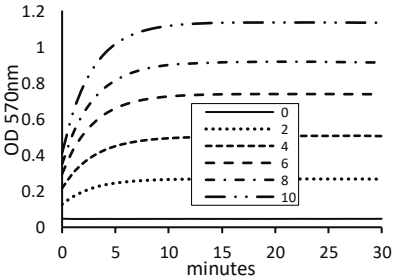
Add 50 µl of the Reaction Mix to each well containing the Pyruvate Standard & test samples Add 50 µl background control mix to background wells.

4. **Measurement:** Monitor the reaction for 30 min. at RT using absorbance (570 nm) or fluorescence (Ex/Em = 535/590 nm) in a microplate reader.

5. Typical Results:

nmol Standard	Standard Raw Values OD		Background Corrected Values OD	
0	0.0475	0.0462	0	0
2	0.2731	0.2607	0.2256	0.2145
4	0.4858	0.4938	0.4383	0.4476
6	0.7139	0.7196	0.6664	0.6734
8	0.9430	0.9533	0.8955	0.9071
10	1.1624	1.1728	1.1149	1.1266





6. Calculation: Subtract 0 Standard reading from all standards. Plot the Pyruvate Standard Curve and determine the slope of the standard curve. This value will be used to calculate pyruvate in unknown test samples. If a background control well was run, subtract background readings from each paired unknown sample. Divide the background corrected sample signal by the slope of the standard curve. This gives the nano- or picomoles of pyruvate in the well. For samples spiked with an internal standard, subtract the unknown sample from the unknown sample spiked with a known amount of pyruvate. The signal obtained is the response of the system for the spike amount. The corrected amount of pyruvate in the well containing unknown alone =
{ signal $_{\text{unknown sample}}$ / [(signal $_{\text{spiked sample}}$ – signal $_{\text{unknown sample}}$) /spike amount] }
To convert from the amount in the wells to the amount of pyruvate in the original samples:

A. nmoles pyruvate in well / µl sample added to well = nmole pyruvate per µl sample.
B. Nmoles pyruvate per µl sample X total volume of sample (original sample + diluent volume) = total nmoles of pyruvate in sample
C. Total nmoles pyruvate in sample / mg tissue (or # of cells or µl serum, etc.) = nmol pyruvate/ mg tissue (cells, etc.)

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