MA-0107

Pyruvate Kinase Activity Assay

(100 wells, Absorbance (OD 570 nm) or Fluorescence (Ex/Em = 535/587 nm), Store at -20°C)

Background Information:

Pyruvate kinase (PK) is a key enzyme in the glycolytic pathway, catalyzing the final step, conversion of phosphoenolpyruvate (PEP) to pyruvate forming ATP. This reaction serves as a key control point, regulating the overall rate of glycolysis and determining the fate of glucose metabolism. PK plays a central role providing energy for cellular processes, as pyruvate is a metabolite common to several pathways. PK also contributes to lactate production under anaerobiosis and influences the balance between oxidative phosphorylation and anaerobic glycolysis, a highly important metabolic choice. AkrivisBio's Pyruvate Kinase Assay provides a simple, sensitive method for determining PK activity, from a variety of sample types down to below 50 µU.

Assay Principle:

1 - Phosphoenolpyruvate + ADP is converted to pyruvate + ATP
2 - Pyruvate is oxidize by pyruvate oxidase, forming hydrogen peroxide
3 - Hydrogen Peroxide is utilized by peroxidase to oxidize ADHP to resorufin possessing intense color (570 nm) and fluorescence (535 nm/ 587 nm)

Assay Components:

Assay Buffer	25 ml	WM	MA-0107A
ADHP Solution	200 µl	Red	MA-0107B
Pyruvate Oxidase/Peroxidase	Lyoph	Green	MA-0107C
PEP/ADP	Lyoph	Purple	MA-0107D
PK Positive Control	Lyoph	Blue	MA-0107E
Pyruvate Standard	100 µl	Yellow	MA-0107F

Storage and Handling:

Store the kit at -20°C. Warm Assay Buffer and ADHP Solution to room temperature before use. Briefly centrifuge vials before opening.
Assay Buffer: Ready to use as supplied. Store at 4°C.
ADHP Solution: Ready to use as supplied. Store at -20°C.
PEP/ADP, Pyruvate Oxidase/Peroxidase: Reconstitute each with 220 µl Assay Buffer. Store at -20°C. Keep on ice while in use. If the assay is to be used repeatedly over a period of time, aliquot the enzyme into convenient portions and store at -20°C to avoid repeated freeze-thaw cycles.
PK Positive Control: Dissolve with 100 µl DI H_2O.Store at -20°C.

Assay Protocol:

1. Standard Curve:
a. Absorbance-based assay: Transfer 10 µl of the Standard to 990 µl of Assay Buffer giving a 0.4 mM solution.
b. Fluorescence-based assay: Dilute the Pyruvate Standard as in the absorbance-based assay, then transfer 10 µl of the diluted standard to 90 µl of Assay Buffer, diluting it another 10X to 40 µM.
c. Transfer 0 – 5 – 10 – 15 – 20 - 25 µl of the standard 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, and 10 nmol/well of the Pyruvate Standard for the absorbance-based, and 0, 200, 400, 600, 800, and 1000 pmol/well for the fluorescence-based assay.
2. Positive Control: Take 2-5 µl and dilute 10X. Absorbance: Add 5-25 µl to a well in a 96 well plate. Fluorescence: Add 1 – 5 µl for the fluorescence-based assay.
3. Sample Preparation: Homogenize tissue (10 mg) or cells (10^6) with 100 µl of Assay Buffer, centrifuge at 16,000 X g for 5 min. Transfer 2 - 50 µl of sample to a 96 well plate. Serum can be added directly into sample wells. Adjust all well volumes to 50 µl/well with Assay Buffer. It is important that all unknown readings fall within the range of the standard curve. If any fall outside this range, dilute and rerun.
Note: Some samples may exhibit a high background. To address this, run those samples in duplicate with one of the paired wells being used as a background control well (omitting the pyruvate oxidase/peroxidase mix from those wells.)

4. Initiate Reaction: Each reaction requires 50 µl of reaction mix. Prepare sufficient reaction mix for the number of wells to be run:

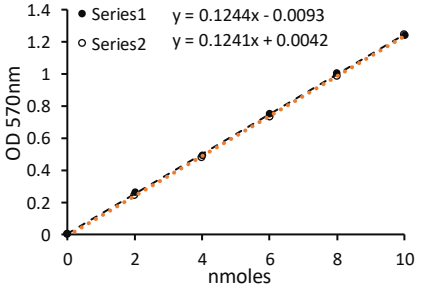
	Reaction Mix	Background Control Mix
Assay Buffer	44 µl	46 µl (47.6 µl for fluorescence-based assay)
PEP/ADP	2 µl	2 µl
Pyruvate Oxidase/Peroxidase	2 µl	-----
ADHP Solution	2 µl (0.4 µl for fluorescence)	2 µl (0.4 µl for fluorescence)

Add 50 µl of the reaction mix to each well containing the pyruvate standard, samples and controls, mix well.

5. Measurement: Monitor the reaction for 30 – 60 min at room temperature, 570 nm for the absorbance-based assay or using Excitation 535 nm/ Emission 587 nm for the fluorescence-based assay. There will likely be an initial lag phase before the enzyme activity is seen at its maximum and a late phase where the rate is slowing due to substrate depletion. Select the time range where the slope is steepest to calculate the enzyme activity.

6. Typical Results:

nmol Standard	Standard Raw Values OD		Background Corrected Values OD	
0	0.0894	0.1106	0	0
2	0.3525	0.3497	0.2631	0.2391
4	0.5806	0.5867	0.4912	0.4761
6	0.8386	0.8430	0.7492	0.7324
8	1.0915	1.0958	1.0021	0.9852
10	1.3318	1.3526	1.2424	1.2420



7. Calculation: Subtract 0 standard readings from all standards. Plot the corrected Standard curve and determine the slope of the Standard Curve. Determine the slope of the enzyme activity in a time period in which the enzyme activity is linear. Divide the slope of the enzyme activity (OD/min or RFU/min) by the slope of the Standard Curve to get enzyme activity in nmoles/min (mU) in the well. To convert to mU in the original sample:
A. Divide the activity (nmol/min) in the well by the volume of sample in µl added to well = activity/µl sample
B. Multiply activity/µl sample by the total volume of sample supernatant obtained after centrifugation in step 3 above = total activity in sample.
C. Divide total activity in the sample by mg tissue (or # of cells or µl serum) used to prepare the sample to get activity/mg tissue (or # of cells etc.)

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48511 Warm Springs Blvd. # 213, Fremont, CA 94539 +1(408)739-9315 contact@akrivisbio.com