Product Information

Pyruvate Kinase Activity Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2223-100 100 assays	Cap Color	Part Number
PK Assay Buffer	25 ml	WM	K2223-C-1
OxiRed™ Probe	200 µl	Red	K2223-C-2
PK Enzyme Mix	Lyophilized	Green	K2223-C-3
PK Substrate Mix	Lyophilized	Purple	K2223-C-4
PK Positive Control	Lyophilized	Blue	K2223-C-5
Pyruvate Standard (100 nmol/µl)	100 µl	Yellow	K2223-C-6

II. Introduction:

Pyruvate kinase (PK) is an important enzyme involved in glycolysis metabolic pathway. PK catalyzes the transfer of a phosphate group from phosphoenolpyruvate (PEP) to ADP, producing ATP and pyruvate. Lack of pyruvate kinase will cause the disease pyruvate kinase deficiency which slows down the process of glycolysis.

The Pyruvate Kinase Activity Colorimetric/Fluorometric Assay Kit provides a sensitive, simple, fast and convenient way for accurate detection of PK activity in various samples (blood, tissues, and culture cells, etc.) based on colorimetric and fluorometric method. In the assay, PEP and ADP were catalyzed by PK to produce ATP and pyruvate, which is subsequently oxidized by pyruvate oxidase to generate fluorescence (Ex/Em = 535/587 nm) and color (λ = 570 nm). The increase in fluorescence intensity or color is proportional to the increase in pyruvate amount. The kit can detect 0.1 mU/ml pyruvate kinase.

III. Reagent Preparation and Storage Conditions:

OxiRed™ Probe: Ready to use as supplied. Allow to come to room temperature before use to melt frozen DMSO. Store at -20℃, protect from light and moisture. Use within two months.

PK Substrate Mix, PK Enzyme Mix: Dissolve with 220 µl diH_2O. Pipette up and down to completely dissolve. Store at -20℃. Use within two months.

PK Positive Control: Dissolve with 100 µl diH_2O. Pipette up and down to completely dissolve. Store at -20℃. Use within two months.

IV. Pyruvate Kinase Assay Protocol:

1. Standard Curve Preparations:

For the colorimetric assay: Dilute the Pyruvate Standard to 1 nmol/µl by adding 10 µl of the Standard to 990 µl of Assay Buffer, mix well.

For the fluorometric assay: Dilute the Pyruvate Standard to 1 nmol/µl as for the colorimetric assay. Then dilute the standard another 10-fold to 0.1 nmol/µl by mixing 10 µl with 90 µl of Pyruvate Assay Buffer. Mix well.

Add 0, 2, 4, 6, 8, 10 µl of the diluted standard into a series of wells. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 2, 4, 6, 8, and 10 nmol/well of the Pyruvate Standard for the colorimetric assay, or 0, 0.2, 0.4, 0.6, 0.8, and 1.0 nmol/well for the fluorometric assay.

2. Sample and Positive Control Preparations: Serum can be directly added into sample wells. Tissues or cells can be extracted with 4 volumes of the Assay Buffer, centrifuge to get clear extract. Add samples directly into 96 well plate, bring volume to 50 µl/well with PK Assay Buffer. We suggest

testing several doses of your sample to ensure the readings are within the linear range. For the positive control (optional), add 5 µl positive control solution to wells (use 0.5 – 2 ul Positive Control for fluorometric assay), adjust volume to 50 µl/well with Assay Buffer.

3. Reaction Mix Preparation: Mix enough reagents for the number of standard and assays to be performed. For each well, prepare a total 50 µl Reaction Mix containing:

	Pyruvate Kinase Measurement	Background Control
Assay Buffer	44 µl	46 µl
Substrate Mix	2 µl	---
Enzyme Mix	2 µl	2 µl
OxiRed™ Probe	2 µl	2 µl

Pyruvate in the sample will generate background. If significant amount of pyruvate is in your sample, the background control should be performed. The background readings are then subtracted from your sample readings.

The fluorometric assay is ~10 times more sensitive than the colorimetric assay. Use 0.4 µl of the probe per reaction to decrease the background reading/increase detection sensitivity significantly.

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

5. Measure OD 570 nm or fluorescence Ex/Em = 535/587 nm at T1 to read A1, measure again at T2 after incubating the reaction at 25℃ for 10 - 20 min (or incubate longer time if the PK activity is low in sample) to read A2, protect from light. The signal increase is due to pyruvate generated by PK, ΔA = A2 – A1.

Note: It is essential to read A1 and A2 in the reaction linear range. It will be more accurate if you read the reaction kinetics. Then choose A1 and A2 in the reaction linear range.

6. Calculation: Subtract 0 standard readings from the standards. Plot the pyruvate standard curve. Apply the ΔA to the standard curve to get B nmol of pyruvate generated between T1 and T2 by PK in the reaction wells. PK calculation:

PK Activity = B /[(T2-T1) x V] x Sample Dilution Factor = nmol/min/ml = mU/mL

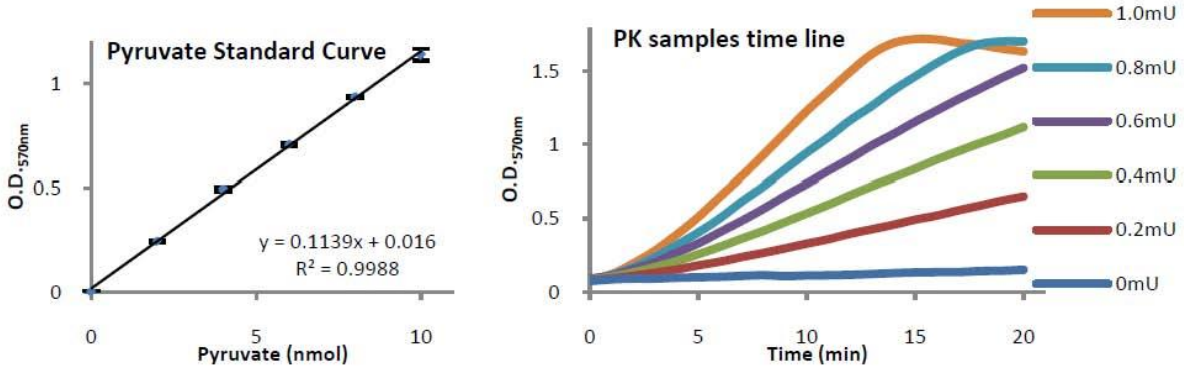
Where: B is the pyruvate amount from pyruvate standard curve (in nmol).

T1 is the time of the first reading (A1) (in min).

T2 is the time of the second reading (A2) (in min).

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

Unit definition: One unit of Pyruvate Kinase is the amount of enzyme that will transfer a phosphate group from PEP to ADP, yielding 1.0 µmol of pyruvate per minute at 25℃.



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General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

For research use only! Not to be used in humans.

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Our promise

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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