3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Pyruvate Kinase Activity Assay Kit

Catalog Number **MAK072**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Pyruvate kinase (PK) is an enzyme that catalyzes the final step of glycolysis, the transfer of a phosphate group from phospho(enol)pyruvate (PEP) to ADP, yielding one molecule of pyruvate and one molecule of ATP. Pyruvate kinase deficiency, due to defects in pyruvate kinase expression or activity, is the second most common cause of hemolytic anemia.

The Pyruvate Kinase Activity Assay Kit provides a simple and direct procedure for measuring pyruvate kinase activity in a variety of biological samples. Pyruvate concentration is determined by a coupled enzyme assay, which results in a colorimetric (570 nm)/ fluorometric (λ_{ex} = 535/λ_{em} = 587 nm) product, proportional to the pyruvate present. One unit of pyruvate kinase is the amount of enzyme that will transfer a phosphate group from PEP to ADP to generate 1.0 μmole of pyruvate per minute at 25 °C.

Components

The kit is sufficient for 100 assays in 96 well plates.

Pyruvate Kinase Assay Buffer Catalog Number MAK072A	25 mL
Fluorescent Peroxidase Substrate, in DMSO Catalog Number MAK072B	0.2 mL
Pyruvate Kinase Enzyme Mix Catalog Number MAK072C	1 vl
Pyruvate Kinase Substrate Mix Catalog Number MAK072D	1 vl
Pyruvate Kinase Positive Control Catalog Number MAK072E	1 vl
Pyruvate Standard, 100 nmole/μL Catalog Number MAK072F	0.1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Pyruvate Kinase Assay Buffer – Allow buffer to come to room temperature before use.

Fluorescent Peroxidase Substrate – Allow reagent to come to room temperature before use. Mix well by pipetting, then aliquot and store, protected from light and moisture, at –20 °C. Upon thawing, the Fluorescent Peroxidase Substrate is ready-to-use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the Fluorescent Peroxidase Substrate 5 to 10-fold with Pyruvate Kinase Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

Pyruvate Kinase Substrate Mix and Pyruvate Kinase Enzyme Mix – Reconstitute each in 220 μL of water. Mix well by pipetting, then aliquot and store at –20 °C. Use within two months of reconstitution.

Pyruvate Kinase Positive Control - Reconstitute in 100 µL of water. Mix well by pipetting, then aliquot and store at –20 °C. Keep cold while in use. Use within two months of reconstitution.

Storage/Stability
The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Pyruvate Standards for Colorimetric Detection
Dilute 10 µL of the 100 nmole/µL Pyruvate Standard with 990 µl of the Pyruvate Kinase Assay Buffer to prepare a 1 nmole/µL standard solution. Add 0, 2, 4, 6, 8, 10 µL of the 1 nmole/µL standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Pyruvate Kinase Assay Buffer to each well to bring the volume to 50 µL.

Pyruvate Standards for Fluorometric Detection
Prepare a 1 nmole/µL standard solution as for the colorimetric assay. Dilute 10 µL of the 1 nmole/µL standard solution with 90 µL of the Pyruvate Kinase Assay Buffer to make a 0.1 nmole/µL standard solution. Add 0, 2, 4, 6, 8, 10 µL of the prepared 0.1 nmole/µL standard solution into a 96 well plate, generating 0 (blank), 0.2, 0.4, 0.6, 0.8, and 1.0 nmole/well standards. Add Pyruvate Kinase Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Both the colorimetric and fluorometric assays require 50 µL of sample for each reaction (well).

Tissue or cells should be rapidly homogenized with 4 volumes of Pyruvate Kinase Assay Buffer. Centrifuge at 15,000 × *g* for 10 minutes to remove insoluble materials. Bring samples to a final volume of 50 µL with Pyruvate Kinase Assay Buffer.

Serum samples can be directly added to wells. Add 1–50 µL samples into wells of a 96 well plate. Bring samples to final volume of 50 µL with Pyruvate Kinase Assay Buffer.

For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the standard curve range.

For the positive control (optional), add 5 µL of the Pyruvate Kinase Positive Control to wells for the colorimetric assay or use 0.5–2 µL of the Positive Control for fluorometric assay. Adjust well volume to 50 µL with Pyruvate Kinase Assay Buffer.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well).
   Note: Pyruvate in the samples will generate a background signal. To remove the effect of pyruvate background, a sample blank may be set up by omitting the Pyruvate Kinase Substrate Mix (see Table 1). The blank sample readings can then be subtracted from the sample readings.

Table 1.
Reaction Mixes

Reagent	Samples and Standards	Blank Sample
Pyruvate Kinase Assay Buffer	44 µL	46 µL
Pyruvate Kinase Substrate Mix	2 µL	–
Pyruvate Kinase Enzyme Mix	2 µL	2 µL
Fluorescent Peroxidase Substrate	2 µL	2 µL

2. Add 50 µL of the appropriate Reaction Mix to each well. Mix well using a horizontal shaker or by pipetting.

3. After 2–3 minutes, take the initial measurement ($T_{initial}$). For colorimetric assays, measure the absorbance at 570 nm $(A_{570})_{initial}$. For fluorometric assays, measure fluorescence intensity ($FLU_{initial}$, λ_{ex} = 535/λ_{em} = 587 nm).

4. Incubate the plate at 25 °C taking measurements (A_{570} or FLU) every 5 minutes. Protect the plate from light during the incubation.

5. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (Colorimetric – 10 nmole/well or fluorometric – 1.0 nmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.

6. The final measurement [$(A_{570})_{final}$ or FLU_{final}] for calculating the enzyme activity would be penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve, see step 5. The time of the penultimate reading is T_{final}.
   Note: It is essential the final measurement falls within the linear range of the standard curve.

Results

Calculations

Correct for the background by subtracting the final measurement [$(A_{570})_{final}$ or FLU_{final}] obtained for the 0 (blank) standard from the final measurement [$(A_{570})_{final}$ or FLU_{final}] of the standards. Plot the pyruvate standard curve.

Note: A new standard curve must be set up each time the assay is run.

Calculate the change in measurement from $T_{initial}$ to T_{final} for samples and sample blanks.

$$\Delta A_{570} = (A_{570})_{final} - (A_{570})_{initial}$$

or

$$\Delta FLU = FLU_{final} - FLU_{initial}$$

Also, subtract the Sample Blank Δmeasurement value from the sample Δmeasurement values. Compare the Δmeasurement value (ΔA_{570} or ΔFLU) of each sample to the standard curve to determine the amount of pyruvate generated by the kinase assay between $T_{initial}$ and T_{final} (B).

The Pyruvate Kinase activity of a sample may be determined by the following equation:

$$\text{PK Activity} = \frac{B \times \text{Sample Dilution Factor}}{(\text{Reaction Time}) \times V}$$

B = Amount (nmole) of pyruvate generated between $T_{initial}$ and T_{final}

Reaction Time = $T_{final} - T_{initial}$ (minutes)

V = sample volume (mL) added to well

PK activity reported as nmole/min/mL = milliunit/mL, where one milliunit (mU) of pyruvate kinase is defined as the amount of enzyme that will transfer a phosphate group from PEP to ADP to generate 1.0 nmole of pyruvate per minute at 25 °C.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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