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Product Information

Pyruvate Assay Kit

Catalog Number **MAK071**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
Pyruvate is a central molecule in metabolism that is used and synthesized through multiple metabolic pathways including glycolysis and the transamination of alanine. Pyruvate may be further oxidized in the citric acid cycle, converted to carbohydrates during gluconeogenesis, or reduced to lactate. High levels of pyruvate are associated with liver disease and genetic disorders. Pyruvate has also been used to stimulate metabolism leading to loss of body weight.

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.

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

Pyruvate Assay Buffer Catalog Number MAK071A	25 mL
Pyruvate Probe in DMSO Catalog Number MAK071B	0.2 mL
Pyruvate Enzyme Mix Catalog Number MAK071D	1 vl
Pyruvate Standard, 100 nmole/µL Catalog Number MAK071E	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
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter

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

Pyruvate Probe Solution – Warm to room temperature to thaw the solution prior to use. Aliquot and store any remaining solution at –20 °C protected from light and moisture. Upon thawing, the Pyruvate Probe is ready-to-use in the colorimetric assay.

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

Pyruvate Enzyme Mix – Reconstitute in 220 µL of Pyruvate Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

Storage/Stability
The kit is shipped on wet ice and 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 Pyruvate Standard (100 nmole/µL) with 990 µl of the Pyruvate 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 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 Assay Buffer to make a 0.1 nmole/µL standard solution. Add 0, 2, 4, 6, 8, 10 µL of the diluted 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 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 can be homogenized in 4 volumes of the Pyruvate Assay Buffer. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material.

Serum samples can be directly added to wells. Serum typically contains 50–100 pmole/µL of pyruvate.

Note: Samples may be deproteinized with a 10 kDa MWCO spin filter prior to addition to the reaction. This step may be necessary if lactate dehydrogenase (LDH) or other enzymes, which react with pyruvate or intermediates of the coupled assay, are present in the samples. LDH may mediate the conversion of pyruvate to lactate.

Bring samples to a final volume of 50 µL with Pyruvate Assay Buffer.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Assay Reaction
1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Volume
Pyruvate Assay Buffer	46 µL
Pyruvate Probe Solution (colorimetric or fluorescence)	2 µL
Pyruvate Enzyme Mix	2 µL

2. Add 50 µL of the Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at room temperature. Protect the plate from light during the incubation.

3. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 587 nm).

Results
Calculations
The background for either assay is the value obtained for the 0 pyrurate standard (blank). Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

Use the values obtained from the appropriate pyruvate standards to plot a standard curve. The amount of pyruvate present in the samples may be determined from the standard curve.
Note: A new standard curve must be set up each time the assay is run.

Concentration of Pyruvate

$$S_a/S_v = C$$

S_a = Amount of pyruvate in unknown sample (nmole) from standard curve
S_v = Sample volume (µL) added to reaction well
C = Concentration of pyruvate in sample

Pyruvate molecular weight: 88.08 g/mole

Sample Calculation
Amount of pyruvate (S_a) = 5.84 nmole (from standard curve)
Sample volume (S_v) = 50 µL

Concentration of pyruvate in sample
5.84 nmole/50 µL = 0.1168 nmole/µL

0.1168 nmole/µL × 88.08 ng/nmole = 10.3 ng/µL

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
	Samples were not deproteinized	Use a 10 kDa MWCO spin filter to deproteinize samples
	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 needed to use 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 Master 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 Master 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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