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Product Information

2-Phosphoglycerate Colorimetric/Fluorometric Assay Kit

Catalog Number **MAK198**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

2-Phosphoglycerate (2PG) is an important intermediate in the glycolysis pathway, a critical mechanism of ATP generation. It is converted to phosphoenol pyruvate (PEP), catalyzed by the enzyme enolase.[1] The consumption of glucose and the rate of glycolysis is upregulated in metastatic cancers, a phenomenon known as the Warburg effect.[2,3] Measurement of intracellular levels of 2PG is a tool for analyzing the glycolytic pathway in cancer cells.

The 2-Phosphoglycerate Colorimetric/Fluorometric Assay Kit is a simple assay that measures 2PG in various samples (ranging from 2–10 nmole/well for the colorimetric assay and from 50–250 pmole/well for the fluorometric assay). 2PG concentration is determined by converting the 2PG to PEP and then to pyruvate. The pyruvate is oxidized, resulting in a colorimetric (570 nm) or fluorometric (λ_{ex} = 535/ λ_{em} = 587 nm) product proportional to the amount of 2PG present. Note: The fluorometric assay is 10–100 times more sensitive than the colorimetric assay.

Components

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

2PG Assay Buffer Catalog Number MAK198A	25 mL
2PG Probe, in DMSO Catalog Number MAK198B	0.2 mL
2PG Enzyme Mix Catalog Number MAK198C	1 vl
2PG Converter Catalog Number MAK198D	1 vl
2PG Developer Catalog Number MAK198E	1 vl
2PG Standard Catalog Number MAK198F	1 vl

Reagents and Equipment Required but Not Provided

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays and black plates for fluorometric assays.
- Spectrophotometric or fluorometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the 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.

2PG Assay Buffer – Store the buffer at –20 °C or 2–8 °C. Allow buffer to come to room temperature before use.

2PG Probe – Store at –20 °C, protected from light and moisture. Warm the solution for 1–2 minutes at 37 °C prior to use to melt the DMSO. Mix well by pipetting. Use within 2 months.`

2PG Enzyme Mix, 2PG Converter, and 2PG Developer – Reconstitute each with 220 µL of 2PG Assay Buffer. Mix well by pipetting. Aliquot and store at –20 °C. Use within 2 months of reconstitution. Keep on ice while in use.

2PG Standard – Reconstitute with 100 µL of water to generate a 100 mM (100 nmole/µL) 2PG Standard Solution. Mix well and store at –20 °C. Use within 2 months of reconstitution. Keep on ice while in use.

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.

2PG Standards for Colorimetric Detection
Dilute 10 µL of the 100 mM (100 nmole/µL) 2PG Standard Solution in 990 µL of water to generate a 1 mM (1 nmole/µL) 2PG Standard Solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM 2PG Standard Solution into a 96 well plate generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add 2PG Assay Buffer to each well to bring the volume to 50 µL.

2PG Standards for Fluorometric Detection
Dilute 25 µL of the 1 mM (1 nmole/µL) 2PG Standard Solution with 975 µL of water to generate a 25 µM (25 pmole/µL) 2PG Standard Solution. Add 0, 2, 4, 6, 8, and 10 µL of the a 25 µM (25 pmole/µL) 2PG Standard Solution into a 96 well plate generating 0 (blank), 50, 100, 150, 200, and 250 pmole/well standards. Add 2PG Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Clear liquid samples can be measured directly.

Tissue samples (10 mg) or cells (1×10^6) can be homogenized in 200 µL of ice cold 2PG Assay Buffer. Centrifuge the samples at 12,000 × *g* for 5 minutes to remove insoluble material. Transfer supernatant to a fresh tube.

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.

Add 1–50 µL of the samples into duplicate wells. Bring samples to a final volume of 50 µL using 2PG Assay Buffer.

For samples exhibiting significant background, especially background caused by pyruvate, include a Sample Blank for each sample by omitting the 2PG Enzyme Mix. The Sample Blank readings can then be subtracted from the sample readings.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1 or Table 2. 50 µL of the appropriate Reaction Mix is required for each reaction (well).

Table 1.
Colorimetric Reaction Mixes

Reagent	Standards and Samples	Sample Blank
2PG Assay Buffer	42 µL	44 µL
2PG Probe	2 µL	2 µL
2PG Enzyme Mix	2 µL	–
2PG Converter	2 µL	2 µL
2PG Developer	2 µL	2 µL

Table 2.
Fluorometric Reaction Mixes

Reagent	Standards and Samples	Sample Blank
2PG Assay Buffer	43.8 µL	45.8 µL
2PG Probe	0.2 µL	0.2 µL
2PG Enzyme Mix	2 µL	–
2PG Converter	2 µL	2 µL
2PG Developer	2 µL	2 µL

Note: The fluorometric assay is 10–100 times more sensitive than the colorimetric assay.

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting. Incubate the plate at room temperature for 40 minutes. Protect the plate from light during the incubation.

3. Measure the absorbance at 570 nm (A_{570}) or the fluorescence (FLU, λ_{ex} = 535/ λ_{em} = 587 nm) with a microplate reader.

Results

Calculations

The background for either assay is the value obtained for the 0 (blank) 2PG Standard. Correct for the background by subtracting the blank standard value from that of the standards and samples. Background values can be significant and must be subtracted from all readings. Use the corrected values (A_{570} or FLU) obtained from the appropriate 2PG Standards to plot a standard curve.

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

Subtract the Sample Blank value from the final Sample reading to obtain the corrected measurement. Using the corrected measurement, determine the amount of 2PG present in the sample from the standard curve.

Concentration of 2PG

$$C = S_a/S_v$$

where:

S_a = Amount of 2PG in unknown sample well (nmole or pmole) from standard curve

S_v = Sample volume (μL) added into the well

C = Concentration of 2PG in sample (nmole/μL or pmole/μL)

2PG molecular weight is 186.06.

Sample Calculation

Amount of 2PG (S_a) = 5.84 nmole (from standard curve)

Sample volume (S_v) = 50 μL

Concentration of 2PG in sample:

$$5.84 \text{ nmole}/50 \text{ μL} = 0.1168 \text{ nmole/μL}$$

$$0.1168 \text{ nmole/μL} \times 186.06 \text{ ng/nmole} = 21.73 \text{ ng/μL}$$

References

1. Scrutton, M.C., and Utter, M.F., The Regulation of Glycolysis and Gluconeogenesis in Animal Tissues. Annu. Rev. Biochem., **37**, 249–302 (1968).
2. Gatenby, R.A., and Gillies, R.J., Why do cancers have high aerobic glycolysis? Nat. Rev. Cancer, **4**, 891–899 (2004).
3. López-Lázaro, M., The Warburg effect: why and how do cancer cells activate glycolysis in the presence of oxygen? Anticancer Agents Med. Chem., **8**, 305–312 (2008)

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 colorimetric assays, use clear plates. For fluorescence assays, use black plates with clear bottoms.
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 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 Reaction Mixes 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 Mixes
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare Reaction Mixes 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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