Technical Bulletin

Phosphoglycerate Mutase Activity Assay Kit (Colorimetric/Fluorometric)

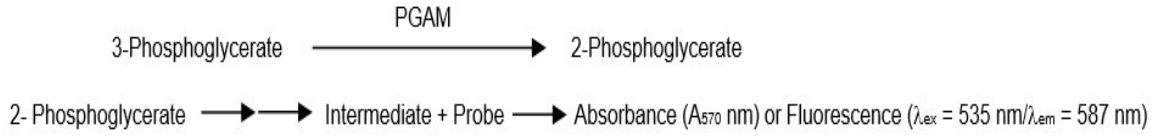
Catalog Number MAK424

Product Description

Phosphoglycerate Mutase (PGAM) is an important enzyme in the glycolytic pathway that catalyzes the reversible conversion of 3-phosphoglycerate to 2-phosphoglycerate. It plays an important role in regulating glycolysis and anabolic activity to promote cancer cell proliferation. Several studies reported that PGAM is upregulated in many human cancers. Inhibition of this enzyme could lead to tumor cell death thereby making PGAM an attractive therapeutic target.

The Phosphoglycerate Mutase Activity Assay Kit can be used to detect its activity in biological samples. The kit utilizes the ability of an active PGAM to catalyze the conversion of 3-Phosphoglycerate to 2-Phosphoglycerate (2-PG), which is subsequently used to generate an intermediate product. The intermediate product stoichiometrically reacts with a probe to generate a measurable color (at 570 nm) or fluorescence (λ_{Ex} = 535 nm/λ_{Em} = 587 nm). This assay kit provides a rapid, simple and sensitive method to detect PGAM activity as low as 0.05 mU in a variety of samples.

The kit is suitable for the measurement of PGAM activity in various tissues (liver, muscle, heart, etc.) and cells (adherent or suspension cells). It may also be used for analysis of glycolytic metabolism in different sample types, the mechanistic studies of various cancers, and for the screening of anti-cancer drugs.



Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- PGAM Assay Buffer 25 mL
  Catalog Number MAK424A
- PGAM Lysis Buffer 25 mL
  Catalog Number MAK424B
- Probe 200 µL
  Catalog Number MAK424C
- PGAM Substrate 1 vial
  Catalog Number MAK424D
- PGAM Cofactor 1 vial
  Catalog Number MAK424E
- PGAM Converter 1 1 vial
  Catalog Number MAK424F
- PGAM Converter 2 1 vial
  Catalog Number MAK424G
- PGAM Developer 1 vial
  Catalog Number MAK424H
- PGAM Positive Control 1 vial
  Catalog Number MAK424I
- 2-PG Standard 1 vial
  Catalog Number MAK424J

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Temperature-controlled multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Refrigerated microcentrifuge capable of RCF ≥ 10,000× g

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

PGAM Assay and PGAM Lysis Buffer: Ready to use. Warm to room temperature prior to use. Store at 2-8 °C.

Probe (in DMSO): Ready to use as supplied. Warm to room temperature prior to use. Store at -20 °C. Use within two months.

PGAM Substrate, PGAM Cofactor, PGAM Converter 1, PGAM Converter 2 and PGAM Developer: Reconstitute each vial with 220 µL of PGAM Assay Buffer. Store at -20 °C. Keep on ice while in use.

PGAM Positive Control: Reconstitute vial with 220 µL of PGAM Assay Buffer. Store at -20 °C. Keep on ice while in use. Avoid repeated freeze-thaw cycles. Use within six months after reconstitution.

2-PG Standard: Reconstitute vial with 100 µL of purified water to generate a 100 mM (100 nmol/µL) 2-PG stock standard solution. Keep on ice while in use. Store at -20 °C. Use within two months after reconstitution.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

1. Homogenize tissue (10 mg) or cells (1×10^6 cells) in 200 µL in ice cold PGAM Lysis Buffer on ice.
2. Centrifuge at 10000 × *g*, 4 °C for 10 minutes to remove cell debris and save the supernatant.
3. Add 1-50 µL of the sample supernatant into two parallel wells designated as Sample and Sample Background Control into a 96-well plate. For unknown samples, it is recommended to test several doses of sample to ensure the readings are within the linear range.
4. Adjust the total volume to 50 µL /well with PGAM Assay Buffer.

Colorimetric Standard Curve Preparation

Prepare a 1 mM 2-PG Standard by diluting 10 µL of the 100 mM 2-PG stock standard solution with 990 µL of purified water, mix well. Prepare 2-PG Standards for colorimetric assay according to Table 1. Mix well.

Table 1.
Preparation of 2-PG Standards for colorimetric assay

Well	1 mM 2-PG Standard	PGAM Assay Buffer	2-PG (nmol/well)
1	0 µL	50 µL	0
2	2 µL	48 µL	2
3	4 µL	46 µL	4
4	6 µL	44 µL	6
5	8 µL	42 µL	8
6	10 µL	40 µL	10

Fluorometric Standard Curve Preparation

Prepare a 0.1 mM 2-PG Standard by diluting 100 µL of the 1 mM 2-PG Standard (see Colorimetric Standard Curve Preparation section) with 900 µL of purified water, mix well. Prepare 2-PG Standards for fluorometric assay according to Table 2. Mix well.

Table 2.
Preparation of 2-PG Standards for fluorometric assay

Well	0.1 mM 2-PG Standard	PGAM Assay Buffer	2-PG (nmol/well)
1	0 µL	50 µL	0
2	2 µL	48 µL	0.2
3	4 µL	46 µL	0.4
4	6 µL	44 µL	0.6
5	8 µL	42 µL	0.8
6	10 µL	40 µL	1

PGAM Positive Control

For Colorimetric Assay: Add 5 µL of reconstituted PGAM Positive Control into desired well(s) and adjust the total volume to 50 µL/well with PGAM Assay Buffer.

For Fluorometric Assay: Dilute the reconstituted PGAM Positive Control 10-fold by adding 5 µL of the reconstituted PGAM Positive Control into 45 µL of PGAM Assay Buffer. Add 5 µL of the diluted PGAM Positive Control into desired well(s). Adjust the total volume to 50 µL /well with PGAM Assay Buffer.

Reaction Mix (for all wells except Sample Background Control)

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µL of the appropriate Reaction Mix according to Table 3. Mix well.

Table 3.
Preparation of Reaction Mix

Reagent	Colorimetric Assay Reaction Mix	Fluorometric Assay Reaction Mix
PGAM Assay Buffer	38 µL	39 µL
PGAM Substrate	2 µL	2 µL
PGAM Cofactor	2 µL	2 µL
PGAM Converter 1	2 µL	2 µL
PGAM Converter 2	2 µL	2 µL
PGAM Developer	2 µL	2 µL
Probe	2 µL	1 µL

2. Add 50 µL of the Reaction Mix into each well containing Standard, Positive Control and Sample(s). Mix well.

Sample Background Control Reaction Mix

1. For each Sample Background Control well, prepare 50 µL of the appropriate Reaction Mix according to Table 4. Mix well.

Table 4.
Preparation of Sample Background Control Reaction Mix

Reagent	Colorimetric Assay Reaction Mix	Fluorometric Assay Reaction Mix
PGAM Assay Buffer	40 µL	41 µL
PGAM Cofactor	2 µL	2 µL
PGAM Converter 1	2 µL	2 µL
PGAM Converter 2	2 µL	2 µL
PGAM Developer	2 µL	2 µL
Probe	2 µL	1 µL

2. Add 50 µL of the Sample Background Control Reaction Mix into each well containing Sample Background Control.

Measurement

Measure the absorbance at 570 nm (A_{570}) or fluorescence (λ_{Ex} = 535 nm/λ_{Em} = 587 nm) in kinetic mode for 20-60 minutes at 37 °C. Incubation time depends on the PGAM Activity in Samples. Measure the absorbance or fluorescence in kinetic mode, and then choose any two time points (T_1 & T_2) in the linear range of the curve. The 2-PG Standard Curve can be read in endpoint mode at the end of the incubation period.

Results

1. Subtract the 0 Standard reading from all readings.
2. Plot the 2-PG Standard Curve.
3. If the Sample Background Control reading is significant, subtract the Sample Background Control reading from the Sample(s).
4. Apply the corrected Sample reading to the 2-PG Standard Curve to get B nmol or B pmol of 2-PG generated during the reaction time ($\Delta T = T_2 - T_1$).
5. To determine the activity of PGAM, use the following equation:

PGAM Activity (nmol/min/µL or pmol/min/µL = mU/µL or µU/µL = U/mL or mU/mL) =

$$\frac{B}{\Delta T \times V} \times DF$$

where

B = 2-PG amount from the Standard Curve (nmol or pmol)

ΔT = Difference between T_2 and T_1 (minutes)

V = Enzyme/Sample volume (µL)

D = Sample Dilution factor (D = 1 for undiluted Samples)

Figure 1.
Typical 2-PG Standard Curve
(colorimetric assay)

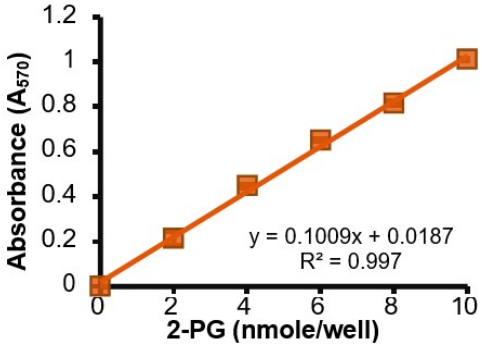


Figure 2.
Typical 2-PG Standard Curve
(fluorometric assay)

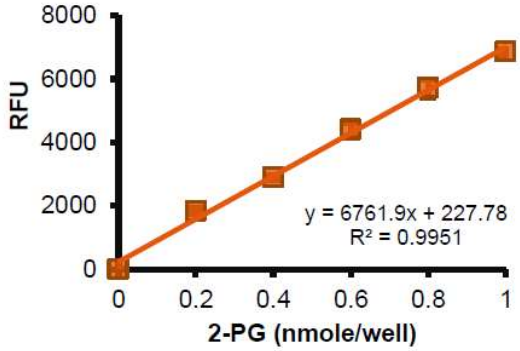
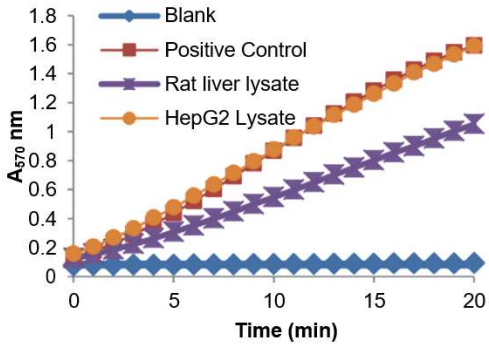


Figure 3.
PGAM activity in rat liver lysate (0.2 μg), HepG2 lysate (0.4 μg) and Positive Control. Assays were performed following the kit protocol.



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