Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Phosphoglycerate Mutase Activity Assay Kit (Colorimetric/Fluorometric)

rev 06/21

(Catalog # K2007-100; 100 assays; Store at -20 °C)

I. Introduction:

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. BioVision's PGAM 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, which is subsequently used to generate an intermediate product. The intermediate product then stoichiometrically reacts with an OxiRed™ probe to generate color (OD 570 nm) or fluorescence (Ex/Em = 535/587 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.

3-Phosphoglycerate $\xrightarrow{\text{PGAM}}$ 2-Phosphoglycerate

2- Phosphoglycerate ⟶⟶ Intermediate + Probe ⟶ Absorbance (OD 570 nm) or Fluorescence (Ex/Em = 535/587 nm)

II. Applications:

- Measurement of PGAM activity in various tissues/cells
- Analysis of glycolytic metabolism in different sample types
- Mechanistic studies of various cancers
- Screening anti-cancer drugs

III. Sample Type:

- Animal tissues: Liver, muscle, heart etc.
- Cell culture: adherent or suspension cells

IV. Kit Contents:

Components	K2007-100	Cap Code	Part Number
PGAM Assay Buffer	25 ml	WM	K2007-100-1
PGAM Lysis Buffer	25 ml	NM	K2007-100-2
OxiRed™ Probe	200 µl	Red	K2007-100-3
PGAM Substrate	1 vial	Amber	K2007-100-4
PGAM Cofactor	1 vial	White	K2007-100-5
PGAM Converter 1	1 vial	Blue	K2007-100-6
PGAM Converter 2	1 vial	Violet	K2007-100-7
PGAM Developer	1 vial	Green	K2007-100-8
PGAM Positive Control	1 vial	Orange	K2007-100-9
2-PG Standard	1 vial	Yellow	K2007-100-10

V. User Supplied Reagents and Equipment:

- dH_2O
- 96-well clear plate with flat bottom
- Temperature-controlled plate reader

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store kit at -20 °C, protected from light. **Briefly centrifuge small vials prior to opening**. Read the entire protocol before performing the assay.

- **PGAM Assay and PGAM Lysis Buffer:** Warm to room temperature (RT) before use. Store at 4 °C.
- **OxiRed Probe (in DMSO):** Ready to use as supplied. Warm to RT before use. Store at -20 °C. Use within two months.
- **PGAM Substrate, PGAM Cofactor, PGAM Converter 1, PGAM Converter 2 & PGAM Developer:** Reconstitute the vial in 220 µl PGAM Assay Buffer. Store at -20 °C. Keep on ice while in use.
- **PGAM Positive Control:** Reconstitute the vial in 220 µl 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 with 100 µl dH_2O to generate 100 mM (100 nmol/µl) stock 2-PG Standard solution. Keep on ice while in use. Store at -20 °C. Use within two months.

VII. PGAM Activity Assay Protocol:

1. Sample Preparation: Homogenize tissue (10 mg) or cells (1 x 10^6) in 200 µl ice cold PGAM Lysis Buffer on ice. Centrifuge at 10000 x *g* and 4 °C for 10 min to remove cell debris and save the supernatant. Add 1-50 µl of the Sample supernatant into two parallel wells designated as **Sample** and **Sample Background Control** of a 96-well clear plate. Adjust the volume to 50 µl/well with PGAM Assay Buffer. **Note:** For Unknown Samples, we suggest testing several doses of your Sample to make sure the readings are within the Standard Curve range.

2. Standard Curve Preparation: For Colorimetric Assay: Dilute 100 mM stock 2-PG Standard to 1 mM 2-PG Standard by adding 10 µl of 100 mM stock 2-PG Standard to 990 µl dH_2O, mix well. Add 0, 2, 4, 6, 8 & 10 µl of 1 mM 2-PG Standard solution into a series of wells. Adjust volume to 50 µl/well with PGAM Assay Buffer to generate 0, 2, 4, 6, 8, and 10 nmol/well of 2-PG Standard.

For Fluorometric Assay: Dilute 1 mM 2-PG Standard to 0.1 mM 2-PG Standard by adding 100 µl of 1 mM 2-PG Standard to 900 µl dH_2O, mix well. Add 0, 2, 4, 6, 8 & 10 µl of 0.1 mM (0.1 nmol/µl) 2-PG Standard solution into a series of wells. Adjust volume to 50 µl/well with PGAM Assay Buffer to generate 0, 0.2, 0.4, 0.6, 0.8, and 1 nmol/well of 2-PG Standard.

3. **PGAM Positive Control: For Colorimetric Assay**, add 5 µl of reconstituted PGAM Positive Control into desired well(s) and adjust the volume to 50 µl/well with PGAM Assay Buffer. **For Fluorometric Assay**, dilute the reconstituted PGAM Positive Control 10 times by adding 5 µl of reconstituted PGAM Positive Control into 45 µl PGAM Assay Buffer. Add 5 µl of the diluted PGAM Positive Control into desired well(s). Adjust the volume to 50 µl/well with PGAM Assay Buffer.
4. **Reaction Mix Preparation:** Mix enough reagents for the number of assays to be performed. For each well, prepare a 50 µl Reaction Mix containing:

	Reaction Mix (Colorimetric)	Reaction Mix (Fluorometric)
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
OxiRed™ Probe	2 µl	1 µl

Prepare **50 µl Background Control Mix** that contains all other reagents like the Reaction Mix but doesn't contain any PGAM Substrate. Add 50 µl of the **Reaction Mix** into each well containing Standards, Positive Control and Sample(s). Add 50 µl of **Background Control Mix** to the Sample Background Control well(s). Mix well. The total reaction volume in each well is 100 µl.

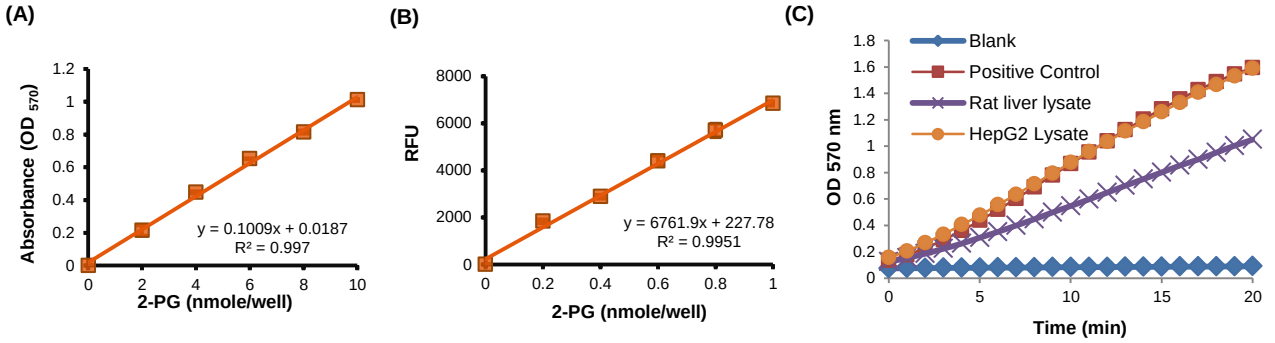
5. Measurement: Measure the absorbance (OD 570 nm) or fluorescence (Ex/Em = 535/587 nm) in kinetic mode for 20-60 min at 37 °C. **Note:** Incubation time depends on the PGAM Activity in Samples. We recommend measuring the absorbance or fluorescence in a kinetic mode, and choosing any two time points (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 30 min incubation).

6. Calculation: Subtract 0 Standard reading from all readings. Plot the 2-PG Standard Curve. If the Sample Background Control reading is significant, subtract the Sample Background Control reading from the Sample(s). 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 ($\Delta T = T_2 - T_1$). To determine the activity of PGAM, use the following equation:

$$\text{Sample PGAM Activity} = \left\{\frac{B}{\Delta T \, X \, V}\right\} XDF = \text{nmol/min/µl or pmol/min/µl = mU/µl or µU/µl = U/ml or mU/ml}$$

Where,

B = 2-PG amount from the Standard Curve (nmol or pmol)
ΔT = Difference between T_2 and T_1 (min)
V = Enzyme/Sample volume (µl)
DF = Sample Dilution factor (D=1 for undiluted Samples)



Figures: (A) 2-PG Standard Curve (colorimetric assay). (B) 2-PG Standard Curve (fluorometric assay). (C) PGAM activity in rat liver lysate (0.2 µg), HepG2 lysate (0.4 µg) and Positive Control. Assays were performed following the kit protocol.

VIII. Related Products:

Phosphoglycerate Kinase Activity Assay Kit (Colorimetric) (K194)
Phosphoglycerate Dehydrogenase (PHGDH) Activity Assay Kit (Colorimetric) (K569)
2-Phosphoglycerate Colorimetric/Fluorometric Assay Kit (K778)
Phosphoglycerate mutase 2, human recombinant (P1103)
Phosphoglycerate mutase 1, Mouse recombinant (P1104)
Phosphoglycerate mutase 1, human recombinant (P1105)

FOR RESEARCH USE ONLY! Not to be used on humans.