ab308240 – Phosphoglycerate Mutase Activity Assay Kit (Colorimetric/Fluorometric)

Detect PGAM activity in biological samples

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: abcam website

Storage and Stability

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.

Materials Supplied

Item	Quantity	Storage Condition
PGAM Assay Buffer	25 ml	4°C
PGAM Lysis Buffer	25 ml	4°C
OxiRed™ Probe (Avoid light)	200 µl	-20°C
PGAM Substrate (Avoid light)	1 Vial	-20°C
PGAM Cofactor	1 Vial	-20°C
PGAM Converter 1	1 Vial	-20°C
PGAM Converter 2	1 Vial	-20°C
PGAM Developer	1 Vial	-20°C
PGAM Positive Control	1 Vial	-20°C
2-PG Standard	1 Vial	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- dH2O
- 96-well clear plate with flat bottom
- Temperature-controlled plate reader

Reagent Preparation

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.

G6PDH Activity Assay Protocol:

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.

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 nmoles/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 nmoles/well of 2-PG Standard.

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.

Reaction Mix Preparation:

Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl of the corresponding (colorimetric or fluorometric) Reaction Mix containing:

	Reaction Mix (Colorimetric)	Reaction Mix (Fluorometric)	Standard/BG Control Mix
PGAM Assay Buffer	38 µl	39 µl	40 or 41 µl
PGAM Substrate	2 µl	2 µl	-
PGAM Cofactor	2 µl	2 µl	2 µl
PGAM Converter 1	2 µl	2 µl	2 µl
PGAM Converter 2	2 µl	2 µl	2 µl
PGAM Developer	2 µl	2 µl	2 µl
OxiRed™ Probe	2 µl	1 µl	1 µl or 2 µl

For Standard wells and Sample Background Control wells, prepare 50 µl of corresponding Standard/BG Control Mix containing all the reagents other than the PGAM Substrate as shown above. Add 50 µl of Standard/BG Control Mix to all Standard wells and Sample Background Control well(s). Add 50 µl of the Reaction Mix into each well containing Sample(s) and Positive Control. Mix well. The total reaction volume in each well including Standards, Sample, Sample Background Control and Positive Control is 100 µl.

Measurement:

Measure the absorbance (OD at 570 nm) or fluorescence (Ex/Em = 535/587 nm) of all sample wells in kinetic mode for 20-60 min at 37 °C.

Δ 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 may be read in Endpoint mode (at the end of 30 min incubation).

Calculation:

Subtract 0 nmole Standard reading from all Standard 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:

Sample PGAM Activity = $\left\{\frac{B}{\Delta T \times V}\right\} \times DF$ **= 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 (in nmole or pmole)
ΔT= Difference between T_2 and T_1 (in minutes)
V= Enzyme/Sample volume (in µl)
DF= Sample Dilution factor (D=1 for undiluted Samples)

Technical Support

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