ab174097

2-Phosphoglycerate Assay Kit (Colorimetric/Fluorometric)

Instructions for Use

For the sensitive and accurate measurement of 2-Phosphoglycerate activity in a variety of samples

This product is for research use only and is not intended for diagnostic use.

Version 4a, Last Updated 30 May 2025

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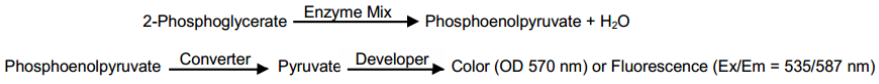
1. Overview

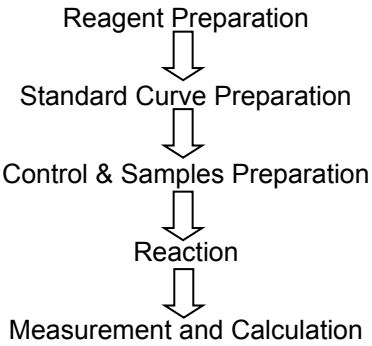
2-phosphoglycerate (2PG) is an important intermediate in the glycolysis pathway. 2-Phosphoglycerate is converted by enolase to phosphoenolpyruvate (PEP) which is a key step from glucose to pyruvate. Aberrant glycolytic metabolism is a highly studied and potentially critical mechanism for ATP generation in cancer cells (The Warburg effect). Measurement of intracellular 2PG levels is a useful tool for analyzing the glycolytic pathway and its relevance to cancer research.

Abcam's 2-Phosphoglycerate Assay kit (ab174097) is a sensitive, fast and easy-to-use kit. In this assay, 2PG is converted by Enzyme Mix to PEP, which is further converted to pyruvate. The pyruvate is oxidized to generate color (OD 570 nm) and fluorescence (Ex/Em = 535/587 nm). The colored product or fluorescence intensity is proportional to 2PG level. This assay kit can detect 2PG level below 20 pmol and can be used for a variety of sample types.

Figure 1: Assay Procedure.



2. Protocol Summary



3. Kits Components

Item	Quantity
Assay Buffer 4	25 mL
2PG Enzyme Mix	1 vial
OxiRed™ Probe	0.2 mL
PEP Converter Mix	1 vial
Developer Mix A	1 vial
2-Phosphoglycerate	1 vial

PLEASE NOTE: Assay Buffer 4 was previously labelled as Assay Buffer IV and 2PG Assay Buffer, and OxiRed™ Probe as OxiRed Probe and 2PG Probe (in DMSO), and Developer Mix A as Development Enzyme Mix I and 2PG Developer (Lyophilized). The composition has not changed.

4. Storage and Stability

Upon arrival, store the kit at -20°C and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Warm Assay Buffer 4 to room temperature before use. Briefly centrifuge all small vials prior to opening.

5. Materials Required, Not Supplied

- 96-well clear plate with flat bottom (for Colorimetric Assay)
- 96-well black plate with flat bottom (for Fluorometric Assay)
- Multi-well spectrophotometer (ELISA reader)
- Multi-channel pipette
- Distilled water

6. Reagents Preparation

1. **2PG Enzyme Mix, PEP Converter Mix and Developer Mix A:**

   Reconstitute each of the components with 220 Assay Buffer 4. Pipette up and down to dissolve completely. Keep on ice while in use. Aliquot and store at -20°C. Avoid repeated freeze/ thaw. All reconstituted components are stable for 2 months at -20ºC.

2. **OxiRed™ Probe:**

   Ready to use as supplied. Warm up solution 1 – 2 min at 37ºC to melt the frozen DMSO before use. Ensure the probe is liquid before using and mix well. Probe can be stored at -20ºC protected from light and moisture. Use within two months.

3. **2-Phosphoglycerate:**

   Reconstitute with 100 µL dH2O to generate a 100 mM (100 nmol/µL) 2-Phosphoglycerate. Keep on ice while in use. Aliquot and store at -20°C. Avoid repeated freeze/ thaw. Reconstituted standard is stable for 2 months at -20ºC.

7. Assay Protocol

1. Sample Preparation and Control Preparation:

a) Cells (starting material: 1 x 10^6 cells)

Harvest cells and spin down briefly and discard supernatant. Resuspend the cell pellet in 200 µL ice cold Assay Buffer 4 and put on ice. Homogenize with a Douncer homogenizer (10 – 15 passes) on ice, or by pipetting up and down using a smaller tip, until efficient lysis is confirmed by viewing the cells under the microscope. Centrifuge homogenate at 12000 rpm for 5 minutes to remove cell debris and collect the supernatant.

Use the supernatant for your subsequent assays. Test different dilutions of the sample to ensure the readings will fall within the linear range of the standard curve.

Add test sample to the wells of a 96-well plate. If volume need is <50 µL, bring it up to 50 µL with Assay Buffer 4.

b) Tissue Sample (starting material: 10mg tissue)

Cut tissue in small pieces, add 200 µL ice cold Assay Buffer 4 and put on ice. Homogenize using a Douncer homogenizer (10 – 15 passes) on ice, until efficient lysis is confirmed, by viewing the cells under the microscope. Spin down the samples and collect the supernatant.

Use the supernatant for your subsequent assays. Test different dilutions of the sample to ensure the readings will fall within the linear range of the standard curve.

Add 1- 50 µL test sample to wells of a 96-well plate. If volume needed is <50 µL, bring it up to 50 µL with Assay Buffer 4.

NOTE:

For unknown samples, we suggest testing several doses of your samples to ensure the readings are within the Standard Curve range.

Control:

If samples contain pyruvate, this will generate background.

For samples having high pyruvate levels, prepare parallel sample well(s) as background control. They will be mixed with Background Control mix (Step 4c)

2. Standard Curve:

For colorimetric assay:

a) Prepare a 1mM 2-Phosphoglycerate by diluting 10 µL of 100 mM 2-Phosphoglycerate into 990 µL dH_2O and mixing well.

b) Using the 1 mM 2-Phosphoglycerate, please prepare a standard curve as follows, in a microplate or microcentrifuge tubes:

2PG 1 mM amount (µL)	Assay Buffer 4 (µL)	Amount in well	END [2PG] IN WELL
0	150	50 µL	0 nmol/well
6	144	50 µL	2 nmol/well
12	138	50 µL	4 nmol/well
18	132	50 µL	6 nmol/well
24	126	50 µL	8 nmol/well
30	120	50 µL	10 nmol/well

Add 50 µL of each standard dilution into a 96-well plate to set up standard. Each dilution has enough amount of standard to set up 2 x duplicates x 50 µL/well.

For fluorometric assay:

Detection sensitivity for fluorometric assays is ~ 10 – 100 fold greater than colorimetric assays, so standards need to be diluted accordingly.

c) Using the 1mM 2-Phosphoglycerate prepared in step **2a**, prepare a 0.025 mM (=25 µM) 2-Phosphoglycerate by adding 25 µL of 1 mM 2-Phosphoglycerate to 975 µL dH2O and mixing well.

d) Using the 25 µM 2-Phosphoglycerate solution, please prepare a standard curve as follows, in a microplate or microcentrifuge tubes:

2PG 25 µM amount (µL)	**Assay buffer 4 (µL)**	**Amount in well**	**END [2PG] IN WELL**
0	150	50 µL	0 pmol/well
6	144	50 µL	50 pmol/well
12	138	50 µL	100 pmol/well
18	132	50 µL	150 pmol/well
24	126	50 µL	200 pmol/well
30	120	50 µL	250 pmol/well

Add 50 µL of each standard dilution into a 96-well plate to set up standard. Each dilution has enough amount of standard to set up 2 x duplicates x 50 µL/well.

3. Reaction Mix:

Prepare Reaction Mix for each reaction containing:

a) COLORIMETRIC REACTION

	Reaction Mix	**Background Control Mix**
Assay Buffer 4	42 µL	44 µL
OxiRed™ Probe	2 µL	2 µL
2PG Enzyme Mix	2 µL	---
PEP Converter Mix	2 µL	2 µL

Developer Mix A	2 µL	2 µL

Mix enough reagents for the number of assays (samples and positive control) to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

	Reaction Mix
Assay Buffer 4	42 µL x (Nb samples + Standards + control +1)
OxiRed™ Probe	2 µL x (Nb samples + Standards + control +1)
2PG Enzyme Mix	2 µL x (Nb samples + Standards + control +1)
PEP Converter Mix	2 µL x (Nb samples + Standards + control +1)
Developer Mix A	2 µL x (Nb samples + Standards + control +1)

We also recommend preparing enough **Background Control Mix** to set up duplicate readings using the same calculation.

b) FLUOROMETRIC REACTION

***Note: For fluorometric Assay, use 1/10 of Probe (0.2 µL/well) to reduce the background.*

	Reaction Mix	**Background Control Mix**
Assay Buffer 4	43.8 µL	45.8 µL

OxiRed™ Probe **	0.2 µL	0.2 µL
2PG Enzyme Mix	2 µL	---
PEP Converter Mix	2 µL	2 µL
Developer Mix A	2 µL	2 µL

Mix enough reagents for the number of assays (samples and positive control) to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

	Reaction Mix
Assay Buffer 4	43.8 µL x (Nb samples + Standards + control +1)
OxiRed™ Probe	0.2 µL x (Nb samples + Standards + control +1)
2PG Enzyme Mix	2 µL x (Nb samples + Standards + control +1)
PEP Converter Mix	2 µL x (Nb samples + Standards + control +1)
Developer Mix A	2 µL x (Nb samples + Standards + control +1)

We also recommend preparing enough **Background Control Mix** to set up duplicate readings using the same calculation.

4. Plate Set up and Detection

a) Add 50 µL of the Reaction Mix to wells

b) Add 50 µL of standard or sample to wells.

c) If necessary, also add 50 µL of Background Control Mix to 50 µL of control well(s). Mix well.

d) Incubate at room temperature for 40 minutes.

e) Measure reading on a microplate reader:

Colorimetric assay: measure OD570nm

Fluorometric assay: measure Ex/Em = 535/587 nm.

8. Data Analysis

Calculations:

a) Correct background by subtracting the value derived from the zero standard from all sample readings.

b) Plot the 2-Phosphoglycerate Standard Curve.

c) If Background Control reading is significantly high, subtract the Background Control reading from sample reading. Apply the corrected sample reading to the 2-Phosphoglycerate Standard Curve to get **B** in nmol (colorimetric) or pmol (fluorometric) [2PG] in the sample wells.

Sample 2PG Concentration (C) = B/V x Dilution Factor = nmol/µL = µmol/mL = mM

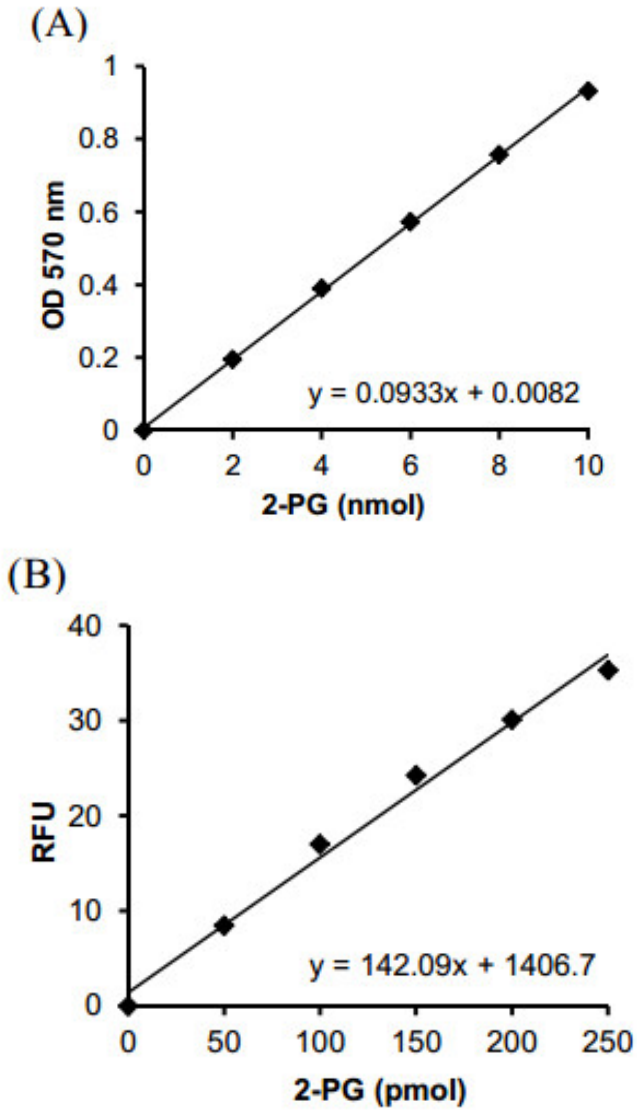
Where:

B = amount of 2PG in the sample well (nmol or pmol)

V = volume of sample used in the reaction (µL)

2PG in samples can also be expressed in nmol/mg of protein

2-Phosphoglycerate molecular weight: 186.06 g/mol


(A)
OD 570 nm
1
0.8
0.6
0.4
0.2
0
y = 0.0933x + 0.0082
0
2
4
6
8
10
2-PG (nmol)
(B)
RFU
40
30
20
10
0
y = 142.09x + 1406.7
0
50
100
150
200
250
2-PG (pmol)

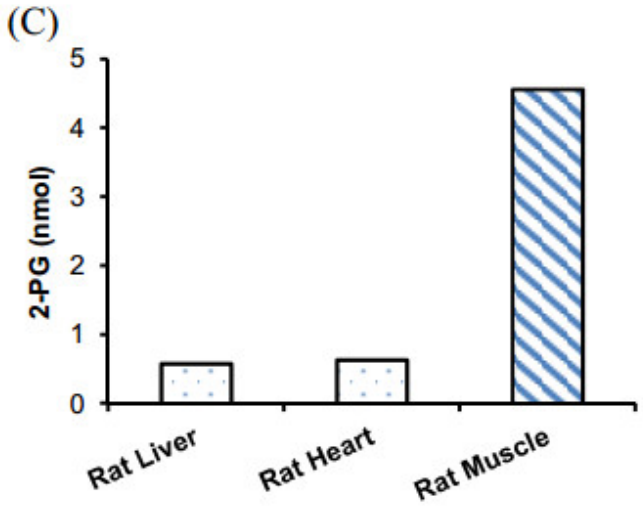


Figure 2: (A) 2-Phosphglycerate/2PG Standard Curve - colorimetric **(B)** 2-Phosphoglycerate Standard Curve - fluorometric **(C)** Measurement of 2PG level in rat liver, heart and muscle lysate (200 µg protein each). Assays were performed according to kit protocol.

9. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

Technical Support

For all technical or commercial enquiries please go to:

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