Version 2a Last updated 17 June 2025

ab273328 Phosphoglycerate Dehydrogenase (PHGDH) Activity Assay Kit (Colorimetric)

View Kit datasheet: https://www.abcam.com/ab273328
(use https://www.abcam.cn/ab273328 for china, or
https://www.abcam.co.jp/ab273328 for Japan)

For the determination of Phosphoglycerate Dehydrogenase (PHGDH) activity in adherent/suspension cells and tissue.

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

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1. Overview

Phosphoglycerate Dehydrogenase (PHGDH) Activity Assay Kit (Colorimetric) (ab273328) provides a quick and easy method for monitoring PHGDH activity in a variety of samples. In this kit, Phosphoglycerate Dehydrogenase converts 3-phosphoglycerate and NAD into 3-phosphohydroxyglerate and NADH respectively. The oxidation of NADH reduces a probe generating a strong, stable absorbance signal (OD: 450 nm).

The assay is simple, sensitive, high–throughput adaptable and can detect Phosphoglycerate Dehydrogenase activity less than 0.1 mU per sample.

2. Protocol Summary

Prepare all reagents, samples and positive controls.

Prepare standards.

Add all samples to the appropriate wells.

Add Reaction Mix and Background Control Mix to the appropriate wells.

Immediately measure absorbance in kinetic mode for 10-60 mins at 37°C.

Determine Phosphoglycerate Dehydrogenase (PHGDH) activity using equation.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
NADH Standard I	1 vial	-20°C
PHGDH Assay Buffer	25 ml	-20°C
Developer Solution III	1 vial	-20°C
PHGDH Positive Control	50 µl	-20°C
3-Phosphoglycerate	1 vial	-20°C

PLEASE NOTE: Developer Solution III was previously labelled as PHGDH Developer Lyophilized, and 3-Phosphoglycerate as PHGDH Substrate Lyophilized. The composition has not changed.

7. Materials Required, Not Supplied

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

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

– Briefly centrifuge small vials at low speed prior to opening.

9.1 **PHGDH Assay Buffer:**

Ready to use as supplied. Bring to room temperature before use. Store at -20°C.

9.2 **3-Phosphoglycerate:**

Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.

9.3 **Developer Solution III:**

Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.

9.4 **NADH Standard I:**

Reconstitute with 400 µl dH_2O to generate 1.25 mM (1.25 nmol/µl) NADH Standard I solution. Keep on ice while in use. Aliquot and store at –20°C. Use within two months.

9.5 **PHGDH Positive Control:**

Keep on ice while in use. Aliquot and store at –70°C. Use within two months.

10. Sample Preparation

Tissue/cell lysate preparation:

10.1 For whole cells or tissue lysate, rapidly homogenize tissue (20mg) or cells (4 x 10^6) with 400 µl ice cold PHGDH Assay Buffer, and place on ice for 10 mins.

10.2 Centrifuge at 10,000 *x g* for 5 mins at 4°C and collect the supernatant. Measure the total protein concentration of cell lysate samples using a BCA assay or equivalent method.

10.3 Use saturated ammonium sulfate to precipitate proteins and remove interferences such as small molecules: Aliquot tissue samples (100 µl) to a clean centrifuge tube, add saturated 4.32 M ammonium sulfate bringing saturation to 65% (1 volume of sample + 2 volumes of 4.32 M ammonium sulfate). Place on ice for 30 mins. Spin down samples at 10,000 *x g* at 4°C for 10 mins, discard the supernatant, and resuspend the pellet back to the original volume with PHGDH Assay Buffer.

10.4 Add 2-50 µl samples into a 96 well clear plate; adjust final volume to 50 µl with PHGDH Assay Buffer.

Δ Note: For unknown samples, we suggest testing several doses of your sample to make sure the readings are within the standard curve range.

Δ Note: If sample exhibits high background, prepare parallel sample well(s) as sample background controls.

11. Standard Curve

– Keep standards on ice while in use.

11.1 Dilute Add 0, 2, 4, 6, 8 and 10 µl of 1.25 mM NADH Standard I into a series of wells in a 96 well plate to generate 0, 2.5, 5, 7.5, 10 and 12.5 nmol/well of NADH Standard.

11.2 Adjust volume to 50 µl/well with PHGDH Assay Buffer.

Standard #	1.25 mM NADH Standard (µL)	PHGDH Assay Buffer (µL)	NADH (nmol/well)
1	0	50	0
2	2	48	2.5
3	4	46	5
4	6	44	7.5
5	8	42	10
6	10	40	12.5

12. Assay Procedure

– Keep on ice while in use.

12.1 **PHGDH Positive Control:** Pipette 5-20 µl of PHGDH Positive Control into wells and adjust final volume to 50 µl with PHGDH assay buffer.

12.2 **Reaction Mix:**
Prepare enough reagents for the number of assays to be performed. For each well, prepare 50 µl of the Reaction mix:

	Reaction mix (µl)	*Background Control mix (µl)
PHGDH Assay Buffer	46	48
Developer Solution III	2	2
3-Phosphoglycerate	2	---

12.3 Add 50 µl of the Reaction mix into Standard, Positive Control and sample wells.

* For samples having high background, add 50 µl of Background Control Mix.

12.4 Measure the plate immediately at 450 nm in kinetic mode for 10-60 mins at 37°C.

Δ Note: Incubation time depends on the PHGDH activity in the samples. We recommend measuring absorbance in kinetic mode and choosing two time points (t1 and t2) in the linear range to calculate the PHGDH activity of the samples. If low activity is expected, longer incubation times may be needed. The NADH standard curve can be read in Endpoint mode (i.e., at the end of incubation time).

13. Calculations

13.1 Subtract the 0 standard reading from all standard readings.
13.2 Plot the NADH standard curve.
13.3 Correct sample background by subtracting the value derived from the background control from all sample readings.
13.4 Calculate the PHGDH activity of the test sample:

$$\Delta OD = A2 - A1.$$

13.5 Apply the ΔOD to the NADH standard curve to get B nmol of NADH generated by PHGDH during the reaction time (Δt = t2 - t1). PHGDH activity can be normalised to the total protein amount in each sample.

$$PHGDH\ activity\ = \frac{B}{(\Delta T\ x\ V)} xD = nmol/min/\mu l = U/ml$$

B = NADH amount from the Standard Curve (nmol)
ΔT = Reaction time ($T_2 - T_1$) (mins)
V = Sample volume added to the reaction well (µl)
D = Dilution factor (D=1 when sample is undiluted)

Unit Definition: One unit of PHGDG is the amount of enzyme that will generate 1.0 µmol of NADH per min at pH 8.4 at 37°.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

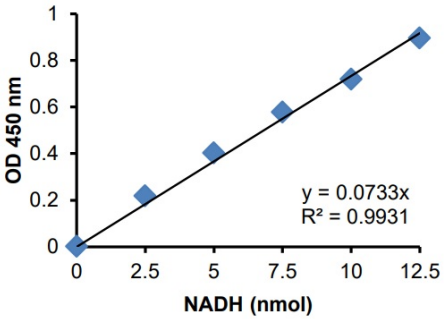

Figure 1. NADH Standard Curve.

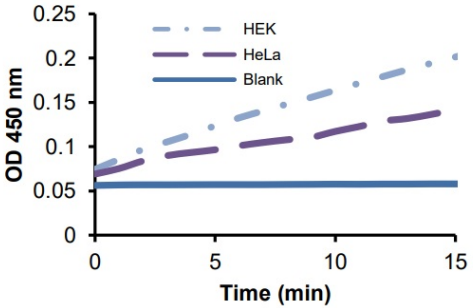

Figure 2. Kinetic measurement of Phosphoglycerate Dehydrogenase activity from various samples.

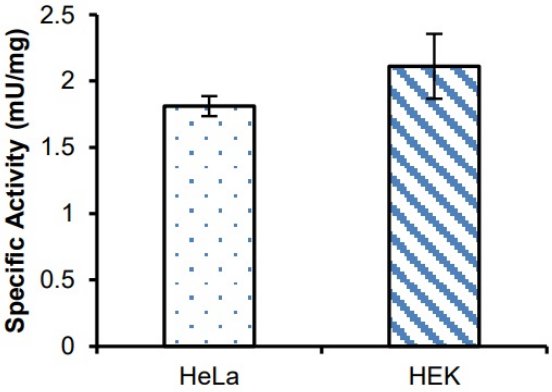

Figure 3. Relative PHGDH Activity was calculated in lysates prepared from HEK cells (4.5 µg per well) and HeLa cells (7.8 µg per well).

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)