Technical Bulletin

Phosphoglycerate Dehydrogenase (PHGDH) Activity Assay Kit (Colorimetric)

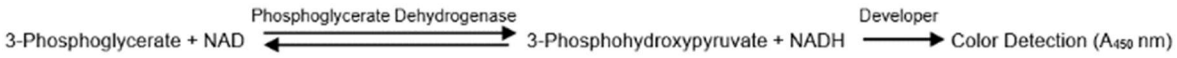
Catalog Number MAK412

Product Description

Phosphoglycerate Dehydrogenase (PHGDH) is the first enzyme that participates in the L-serine synthesis pathway. PHGDH catalyzes the reversible reaction that converts 3-phosphoglycerate into 3-phosphohydroxyglycerate and the NADH-dependent reduction of α-ketoglutarate into D-2-hydroxyglutarate (D-2HG). In humans, PHGDH deficiency causes severe health problems including congenital microcephaly, psychomotor retardation, and intractable seizure. Studies have shown increased PHGDH activity in a wide variety of cancers, particularly in breast and melanoma tumors. Therefore, the measurement of PHGDH activity has become of great importance in searching potential therapeutic agents against cancer.

The Phosphoglycerate Dehydrogenase (PHGDH) Activity Assay kit provides a quick and easy method for monitoring PHGDH activity in a variety of samples. In this assay, 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 at 450 nm. The assay is simple, sensitive, and suitable for high–throughput applications. This method can detect phosphoglycerate dehydrogenase activity less than 0.1 mU per sample.

The kit is suitable for the measurement of PHGDH activity in various tissues (liver, kidney, etc.) and cells (adherent or suspension cells), and for the analysis of de novo L-serine biosynthesis.



Components

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

- PHGDH Assay Buffer — 25 mL
  Catalog Number MAK412A
- PHGDH Substrate — 1 vial
  Catalog Number MAK412B
- PHGDH Developer — 1 vial
  Catalog Number MAK412C
- NADH Standard — 1 vial
  Catalog Number MAK412D
- PHGDH Positive Control — 50 µL
  Catalog Number MAK412E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
- 96-well clear flat-bottom plate. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric multiwell plate reader
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Ammonium sulfate saturated solution (4.32M), prepared from Ammonium sulfate (Catalog Number A4418 or equivalent)

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 kit at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

PHGDH Assay Buffer: Warm to room temperature prior to use. Store at 2-8 °C. Chill an appropriate amount of PGHDH Assay Buffer for use in Sample Preparation.

PHGDH Substrate: Reconstitute vial with 220 µL of purified water. Pipette up and down to dissolve completely. Store at -20 °C. Use within two months of reconstitution.

PHGDH Developer: Reconstitute vial with 220 µL of purified water. Pipette up and down to dissolve completely. Store at -20 °C. Use within two months of reconstitution.

NADH Standard: Reconstitute vial with 400 µL of purified water to generate a 1.25 mM (1.25 nmol/µL) NADH Standard solution. Keep on ice while in use. Aliquot and store at -20 °C. Use within two months of reconstitution.

PHGDH Positive Control: Keep on ice while in use. Aliquot and store at **-80 °C** (**not** -20 °C). Use within two months.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

1. For whole cells or tissue lysate, rapidly homogenize tissue (20 mg) or cells (4×10^6 cells) with 400 µL of ice-cold PHGDH Assay Buffer, and place on ice for 10 minutes.
2. Centrifuge at 10,000 × *g* for 5 minutes at 4 °C and collect the supernatant.
3. Use saturated ammonium sulfate to precipitate proteins and remove interferences such as small molecules:
   a. Aliquot 100 µL of the cell or tissue supernatant to a clean centrifuge tube.
   b. Add 200 µL of saturated 4.32 M ammonium sulfate (not included) bringing saturation to 65% (1 volume of sample + 2 volumes of 4.32 M ammonium sulfate).
4. Place on ice for 30 minutes.
5. Spin down samples at 10,000 × *g* for 10 minutes at 4 °C.
6. Discard the supernatant and resuspend the pellet back to the original volume with PHGDH Assay Buffer.
7. Add 2-50 µL of sample into a 96-well clear plate. For unknown samples, test different amounts of sample to ensure the readings are within the Standard Curve range.
8. Adjust the total volume of each Sample (S) to 50 µL/well with PHGDH Assay Buffer.

Positive Control

To desired well(s), pipette 5-20 µL of PHGDH Positive Control and adjust the total volume to 50 µL/well with PHGDH Assay Buffer.

Sample Background Control (SBC)

Running a sample background control allows for the correction of samples with high background. Use the same amount of tissue/cell homogenate as in the Sample well(s). Adjust the total volume to 50 µL/ well with PHGDH Assay Buffer.

Standard Curve Preparation

Using the 1.25 mM NADH Standard solution, prepare NADH Standards according to Table 1.

Table 1.
Preparation of NADH Standards

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

Reaction Mixes

1. Mix enough reagents for the number of assays to be performed.
   a. For each well containing Sample (S), Positive Control, or Standard, prepare 50 µL of Reaction Mix according to Table 2. Mix well.
   b. For each Sample Background Control well (SBC), prepare 50 µL of Background Control Mix according to Table 2. Mix well.

Table 2.
Preparation of Reaction Mix

Reagent	Reaction Mix	Background Control Mix
PHGDH Assay Buffer	46 µL	48 µL
PHGDH Developer	2 µL	2 µL
PHGDH Substrate	2 µL	-

2. Add 50 µL of the Reaction Mix to each Sample (S), Positive Control, and Standard wells.
3. Add 50 µL of the Background Control Mix to each Sample Background Control (SBC) well. Mix well.

Measurement

Measure the plate immediately at 450 nm (A_{450}) in kinetic mode for 10-60 minutes at 37 °C. Incubation time depends on the PHGDH activity in 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).

Results

1. Subtract the 0-Standard reading from all Standard readings.
2. Plot the NADH Standard curve.
3. Choose two time points (T_1 and T_2) in the linear range to calculate the PHGDH activity of the Sample(s).
4. Correct sample background by subtracting the value derived from the Sample Background Control (SBC) from all Sample (S) readings.
5. Calculate the change in A_{450} for the Sample after correction for sample background: $\Delta A_S = A_{T2\ Sample} - A_{T1\ Sample}$

6. Apply the ΔA_S to the NADH Standard curve to determine B nmol of NADH generated by PHGDH during the reaction time ($\Delta T = T_2 - T_1$).

   Sample PHGDH Activity (nmol/min/μL or mU/μL or U/mL) =

   $[B/(\Delta T \times V)] \times D$

where:

B = NADH amount from standard curve (nmol)
ΔT = Reaction time ($T_2 - T_1$) (minutes)
V = Sample volume added into reaction well (μL)
D = Sample Dilution factor (if applicable; D = for undiluted Samples)

Unit Definition: One unit of Phosphoglycerate Dehydrogenase is the amount of enzyme that will generate 1.0 μmol of NADH per minute at pH 8.4 at 37 °C.

Figure 1.
Typical NADH Standard Curve.

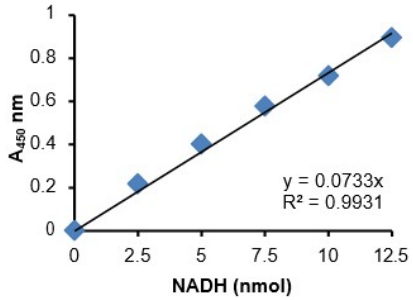


Figure 2.
Kinetic measurement of Phosphoglycerate Dehydrogenase activity in HeLa and HEK cells.

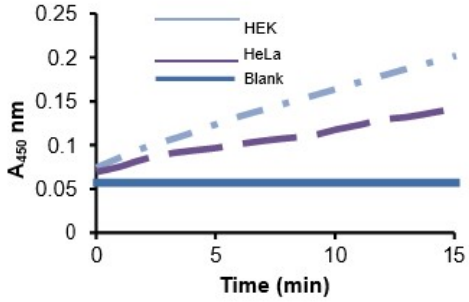
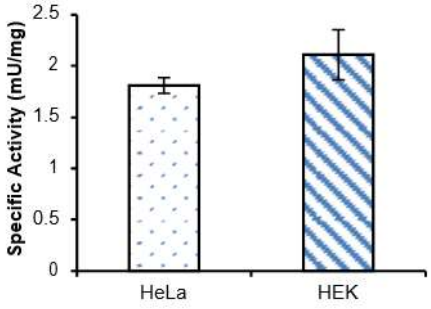


Figure 3.
Relative PHGDH Activity was calculated in lysates prepared from HEK cells (4.5 μg) and HeLa cells (7.8 μg). Assays were performed following kit protocol.



Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Technical Service

Visit the tech service page on our web site at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.