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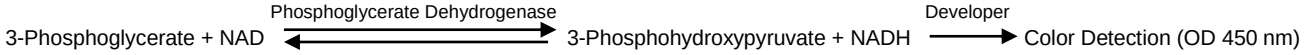
Phosphoglycerate Dehydrogenase (PHGDH) Activity Assay Kit (Colorimetric)

11/18

(Catalog # K569-100; 100 assays; Store at -20°C)

I. Introduction:

Phosphoglycerate Dehydrogenase (PHGDH: EC 1.1.1.95) 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. Recent 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. BioVision's Phosphoglycerate Dehydrogenase Assay kit 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.



II. Application:

- Measurement of PHGDH activity in various tissues/cells
- Analysis of *de novo* L-serine biosynthesis

III. Sample Type:

- Animal tissues: Liver and kidney, etc.
- Cell culture: Adherent or Suspension Cells

IV. Kit Contents:

Components	K569-100	Cap Code	Part Number
PHGDH Assay Buffer	25 ml	WM	K569-100-1
PHGDH Substrate	1 vial	Blue	K569-100-2
PHGDH Developer	1 vial	Red	K569-100-3
NADH Standard	1 vial	Yellow	K569-100-4
PHGDH Positive Control	50 µl	Orange	K569-100-5

V. User Supplied Reagents and Equipment:

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

VI. Storage, Handling and Reagent Preparation:

Store kit at -20°C, protected from light. Warm PHGDH Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening.

- **PHGDH Assay Buffer:** Warm to room temperature before use. Store at 4°C.
- **PHGDH Substrate:** Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **PHGDH Developer:** Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **NADH Standard:** Reconstitute with 400 µl dH_2O to generate 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.
- **PHGDH Positive Control:** Keep on ice while in use. Aliquot and store at **–70°C**. Use within two months.

VII. PHGDH Activity Assay Protocol:

1. **Sample Preparation:** For whole cells or tissue lysate, rapidly homogenize tissue (20 mg) or cells (4 x 10^6) with 400 µl ice cold PHGDH Assay Buffer, and place on ice for 10 minutes. Centrifuge at 10,000 x *g* for 5 min at 4°C and collect the supernatant. 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 (BioVision Cat. # 7096) 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. Add 2-50 µl samples into a 96 well clear plate; adjust final volume to 50 µl with PHGDH Assay Buffer. For PHGDH Positive Control, pipette 5-20 µl of PHGDH Positive Control into wells and adjust final volume to 50 µl with PHGDH assay buffer.
   Notes:
   a. For unknown samples, we suggest testing several doses to ensure the readings are within the standard curve range.
   b. If sample exhibits high background, prepare parallel sample well(s) as sample background controls.
2. **NADH Standard Curve:** Add 0, 2, 4, 6, 8 and 10 µl of 1.25 mM NADH Standard 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. Adjust volume to 50 µl/well with PHGDH Assay Buffer.
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

	Reaction Mix	Background Control Mix*
PHGDH Assay Buffer	46 µl	48 µl
PHGDH Developer	2 µl	2 µl
PHGDH Substrate	2 µl	----

Add 50 µl of the Reaction Mix to each well containing the Standard, Positive Control and test samples.
*For samples having high background, add 50 µl of Background Control Mix.

4. **Measurement:** Measure the plate immediately at 450 nm in kinetic mode for 10-60 min at 37°C.

   Note: Incubation time depends on the PHGDH activity in the samples. We recommend measuring absorbance in kinetic mode, and choosing two time points (t_1 & t_2) 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).

5. **Calculation:** Subtract the 0 standard reading from all standard readings. Plot the NADH standard curve. Correct sample background by subtracting the value derived from the background control from all sample readings. Calculate the PHGDH activity of the test sample: $\Delta OD = A_2 - A_1$. Apply the ΔOD to the NADH standard curve to get B nmol of NADH generated by PHGDH during the reaction time ($\Delta t = t_2 - t_1$).

Sample PHGDH Activity = B/(Δt X V) x D = nmol/min/µl = mU/µl = U/ml

Where: **B** is the NADH amount from standard curve (nmol)
ΔT is the reaction time (min)
V is the sample volume added into the reaction well (µl)
D is the dilution factor

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

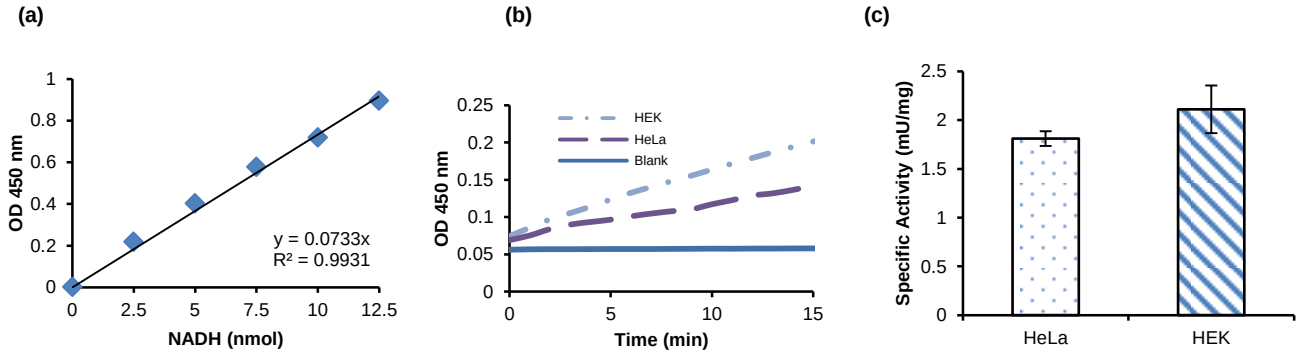


Figure: (a) NADH Standard Curve. (b) Kinetic measurement of Phosphoglycerate Dehydrogenase activity from various samples. (c) Relative PHGDH Activity was calculated in lysates prepared from HEK cells (4.5 µg) and HeLa cells (7.8 µg). Assays were performed following kit protocol.

VIII. RELATED PRODUCTS

Glucose and Sucrose Assay Kit (K616)
Pyruvate Colorimetric /Fluorometric Assay Kit (K609)
Pyruvate Dehydrogenase Activity Assay Kit (K679)
Glucose-6-Phosphate Dehydrogenase Assay Kit (K757)
Phosphoglucomutase Assay Kit (K774)
Glucose Dehydrogenase Activity Assay Kit (K786)
Glucose Uptake Colorimetric Assay (K676)
Glucose Uptake Fluorometric Assay (K666)
Glucose-6-Phosphate Assay Kit (657)
Hexokinase Assay Kit (K789)
Phosphoglucose Isomerase Assay Kit (K775)
Glucose-1-Phosphate Assay Kit (K697)

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