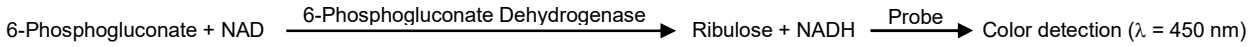
6-Phosphogluconic Acid (6-PGA) Assay Kit (Colorimetric)

(Catalog #BN00494; 100 assays; Store at -20°C)

I. Introduction:

6-Phosphogluconate (6-PGA) is an intermediate of both Pentose Phosphate Pathway (PPP) and Entner-Doudoroff Pathway. It is produced by the hydrolysis of 6-Phosphogluconolactone, catalyzed by 6-Phosphogluconolactonase. In the Pentose Phosphate Pathway, 6-PGA is utilized by 6-Phosphogluconate Dehydrogenase to generate ribulose-5-Phosphate and NADPH. These products are important for nucleic acid synthesis and various anabolic processes. In Prokaryotes, 6-Phosphogluconate is the main metabolite of Entner-Doudoroff pathway, and is converted into Pyruvate using both 6-Phosphogluconate Dehydratase and 2-Keto-3-Deoxyphosphogluconate aldolase. Recent studies show that long-term exposure to glucose perturbs the Pentose Phosphate Pathway, causes significant accumulation of 6-Phosphogluconate and impairs beta cell function. Measurement of 6-Phosphogluconate levels therefore is important for evaluating Pentose Phosphate Pathway, developing therapeutic approaches for diabetes research, and analyzing the Entner-Doudoroff Pathway in bacteria. Assay Genie's 6-Phosphogluconate assay kit can be used with a variety of sample types. In this assay, 6-Phosphogluconate is converted to Ribulose-5-Phosphate by 6-Phosphogluconate Dehydrogenase in the presence of NAD, to form NADH, which reduces a probe and generates strong absorbance at 450 nm. This 6-Phosphogluconate Assay Kit is simple, sensitive & easy to use and can detect 6-Phosphogluconate levels lower than 20 µM.



II. Application:

- Measurement of 6-Phosphogluconic Acid in various tissues/cells.
- Analysis of Pentose Phosphate Pathway and Entner-Doudoroff Pathway.

III. Sample Types:

- Tissues: e.g. Liver, Kidney, Heart
- Adherent or Suspension Cells: e.g. HeLa, Jurkat cells

IV. Kit Contents:

Components	BN00494	Cap Code	Part Number
6-PGA Assay Buffer	25 ml	WM	BN00494-1
6-PGA Enzyme	1 vial	Green	BN00494-2
6-PGA Substrate Mix	1 vial	Red	BN00494-3
6-PGA Standard	1 vial	Yellow	BN00494-4

V. User Supplied Reagents and Equipment:

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

VI. Storage and Handling:

Store the kit at –20°C, protected from light. Warm all Buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

VII. Reagent Preparation and Storage Conditions:

- **6-PGA Enzyme**: Reconstitute with 220 µl 6-PGA Assay Buffer. Pipette up and down to dissolve completely. Keep on ice while in use. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Stable for two months after reconstitution at -20°C.
- **6-PGA Substrate Mix**: Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Stable for 2 months after reconstitution at -20°C.
- **6-PGA Standard:** Reconstitute with 100 µl dH_2O to generate 100 mM (100 nmol/µl) 6-PGA Standard solution. Keep on ice while in use. Store at –20°C. Use within two months.

VIII. Assay Protocol:

1. **Standard Curve Preparation:** Dilute the 6-PGA standard to 1 mM (1 nmol/µl) by adding 10 µl of 100 mM 6-PGA Standard to 990 µl dH_2O & mix well. Add 0, 2, 4, 6, 8, 10 µl of the 1 mM 6-PGA Standard into a 96 well plate to generate 0, 2, 4, 6, 8, and 10 nmol/well of 6-PGA standard. Adjust the volume to 50 µl/well with Assay Buffer.
2. **Sample Preparation:** Tissues (~10 mg) or Cells (~1 $X10^7$) should be rapidly homogenized with 100 µl ice cold 6-PGA Assay Buffer for 5 minutes on ice. Centrifuge at 10000 x g, 4ºC for 5 min. Collect the supernatant. Add 1-50 µl sample per well and adjust the final volume to 50 µl with 6-PGA Assay Buffer.

   Notes:

   A. For unknown samples, we suggest testing several doses of your samples to ensure the readings are within the linear range of the standard curve.

   B. If the samples are not clear, they need to be spin filtered either using 0.22 µm spin column or our 10 Kd spin column with the added benefit of removal of potential interfering enzyme activity. Use the flow through for measurement.

C. NADH in samples will generate a background. Background can be corrected for by making a background control mix omitting the 6-PGA Enzyme in the reaction.

3. **Reaction Mix:** Mix enough reagents for the number of assays (samples and standards) to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	Background Control Mix
6-PGA Assay Buffer	46 µl	48 µl
6-PGA Enzyme	2 µl	----
6-PGA Substrate Mix	2 µl	2 µl

Add 50 µl of the Reaction Mix to each well containing the Standard and test samples and 50 µl of Background Control mix to each well containing the Background Control sample. Mix well.

4. **Measurement:** Incubate for 60 min at 37°C and measure the absorbance at OD_{450nm}.

5. **Calculation:** Subtract the 0 standard reading from all standard readings. Plot the 6-PGA standard curve. Correct the sample background by subtracting the value derived from the background control from all sample readings. Apply the corrected sample reading to standard curve to get 6-Phosphogluconate amount in the sample wells.

The 6-Phosphogluconate concentration in the sample:

C= B/V x D = nmol/µL = mmol/L = mM

Where: **B** = the amount of 6-Phosphogluconic Acid from the standard curve (nmol)
V = the sample volume added into reaction well (µl)
D = Sample Dilution Factor
6-Phosphogluconic Acid: MW: 276.135 g/mol

Sample 6-Phosphogluconic Acid concentration can also be expressed in nmol/mg or µmol/g of sample.

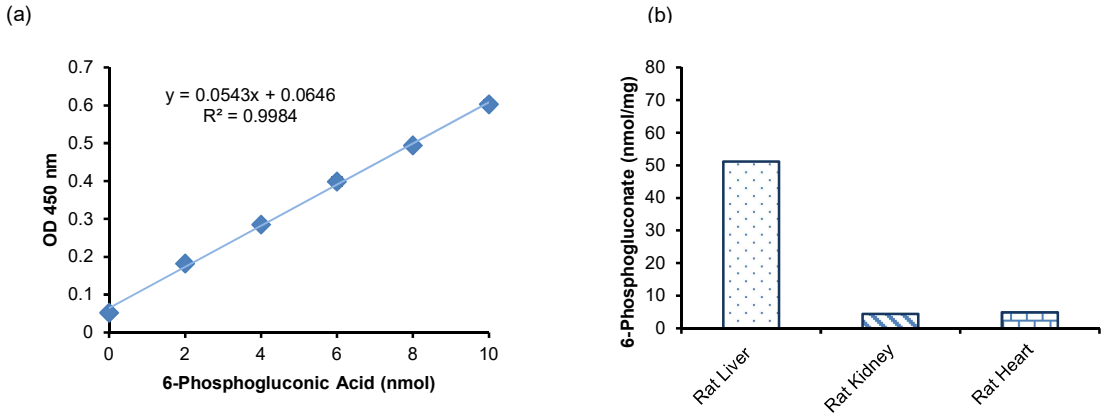


Figure. 6-Phosphogluconate standard curve, n=3 (a). Measurement of 6-Phosphogluconate in the lysates from Rat Liver (160 µg), Rat Kidney (120 µg), and Rat Heart (60 µg). (b). Assays were performed following kit protocol.

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