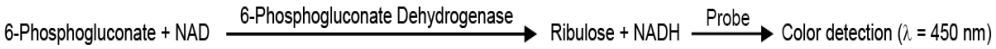
6-Phosphogluconic Acid (6-PGA) Assay Kit

PromoKine

Colorimetric assay for sensitive measurement of 6-Phosphogluconate (6-PGA) levels in cells and tissues

Instruction Manual

Catalog Number	PK-CA577-K217
Description	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. PromoKine'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. 
Quantity	100 assays
Applications	• Measurement of 6-Phosphogluconic Acid in various tissues/cells • Analysis of Pentose Phosphate Pathway and Entner-Doudoroff Pathway
Sample Type	• Tissues: e.g. Liver, Kidney, Heart • Adherent or Suspension Cells: e.g. HeLa, Jurkat cells

Components	Quantity	Color Code
6-PGA Assay Buffer	25 ml	WM
6-PGA Substrate Mix (lyophilized)	1 vial	Red
6-PGA Standard (lyophilized)	1 vial	Yellow
6-PGA Enzyme (lyophilized)	1 vial	Green

User Supplied Reagents & Equipment	• 96-well flat-bottom clear plate • Multi-well spectrophotometer (ELISA reader)
Storage and Reagents Preparation	Store kit at –20°C, protected from light. Warm all buffers to room temperature before use. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay. • **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.
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 and 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 x 10^7) should be rapidly homogenized with 100 µl ice cold 6-PGA Assay Buffer for 5 minutes on ice. Centrifuge at 10000 x g, 4°C for 5 minutes. Collect the supernatant. Add 1-50 µl sample per well and adjust the final volume to

50 µl with 6-PGA Assay Buffer. Note:

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 (Cat.No. PK-CA577-1997) 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 minutes at 37°C and measure the absorbance at OD450nm.

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.

Intended Use

For in vitro research use only. Not for diagnostic or therapeutic procedures.

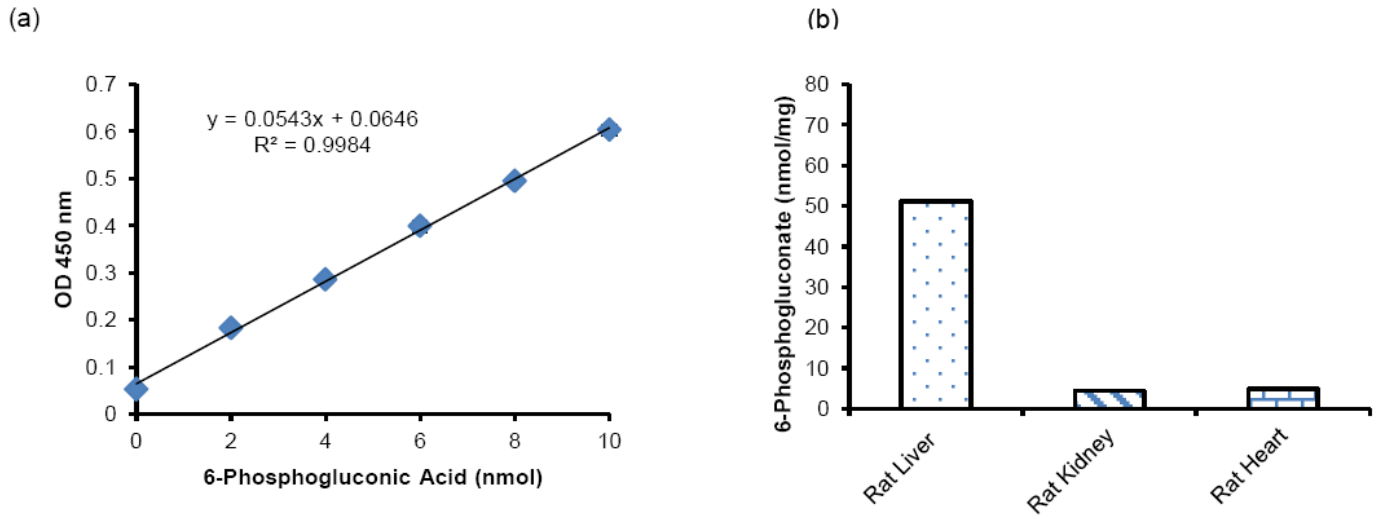


Figure: 6-Phosphogluconate standard curve, n=3 (a). Measurement of 6-Phosphogluconate in the lysates from Rat Liver (160 µg), Rat Kidney (120 µg), and Rat Heart (60 µg). (b). Assays were performed following kit protocol.

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