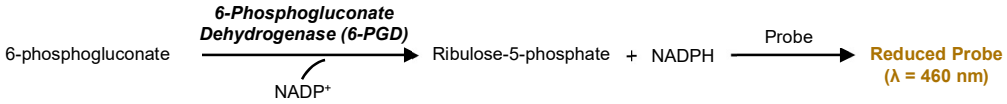
6-Phosphogluconate Dehydrogenase Activity Colorimetric Assay Kit (BN00769)

(Catalog # BN00769; 100 assays; Store at -20°C)

I. Introduction:

6-Phosphogluconate dehydrogenase (6-PGD, EC 1.1.1.44) is an enzyme in the pentose phosphate pathway that catalyzes the oxidative decarboxylation of 6-phosphogluconate to ribulose-5-phosphate with simultaneous reduction of $NADP^+$ to NADPH. Ribulose-5-phosphate is used for production of nucleotides and nucleic acids and can also be reversibly converted to glyceraldehyde-3-phosphate and fructose-6-phosphate which can be used in the glycolysis pathway. It also has a vital role in production of NADPH, which is utilized as a reducing agent in numerous biosynthetic pathways and is important in prevention of oxidative damage. In humans, 6-PGD deficiency is an autosomal hereditary disorder that can result in depletion of NADPH in red blood cells. As NADPH is required for regeneration of the cellular antioxidant glutathione in red blood cells, 6-PGD deficiency increases the risk of hemolytic anemia in situations of oxidative stress. On the other hand, inhibition of 6-PGD has been linked to anti-cancer activity by decreasing RNA biosynthesis and increasing the buildup of reactive oxygen species in tumor cells. Assay Genie's 6-PGD assay kit provides a quick, sensitive and easy way for measuring 6-PGD activity in various biological samples. In this assay, 6-PGD converts 6-phosphogluconate into an intermediate, generating NADPH, which subsequently reduces a colorless probe into a strongly colored product detectable by absorbance at 460 nm. The assay is high-throughput adaptable and can detect less than 0.05 mU of 6-PGD activity.



II. Applications:

- Measurement of 6-phosphogluconate dehydrogenase activity in various tissues/cells.
- Analysis of pentose phosphate pathway.

III. Sample Types:

- Animal tissues: liver, spleen, heart etc.
- Cultured cells (adherent or suspension cells).

IV. Kit Contents:

Components	BN00769	Cap Code	Part Number
6-PGD Assay Buffer	25 ml	WM	BN00769-1
6-PGD Substrate	1 vial	Blue	BN00769-2
6-PGD Developer	1 vial	Red	BN00769-3
NADPH Standard	1 vial	Yellow	BN00769-4
6-PGD Positive Control	1 vial	Violet	BN00769-5

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **6-PGD Assay Buffer:** Warm to room temperature before use. Store at either 4°C or -20°C.
- **6-PGD Substrate:** Reconstitute with 220 µl dH_2O. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.
- **6-PGD Developer:** Reconstitute with 900 µl dH_2O. Gently pipette up and down to dissolve the pellet completely (do not vortex). Aliquot and store at -20°C. Keep on ice while in use. Use within two months.
- **NADPH Standard:** Reconstitute with 200 µl dH_2O to generate 1 mM NADPH Standard solution. Aliquot and store at –20°C. Keep on ice while in use. Use within two months.
- **6-PGD Positive Control:** Reconstitute with 20 µl 6-PGD Assay Buffer and mix thoroughly. Aliquot and store at –20°C. Use within two months. Keep on ice while in use.

VII. 6-Phosphogluconate Dehydrogenase Assay Protocol:

1. **Sample Preparation:** Rapidly homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µl ice-cold 6-PGD Assay Buffer, and keep on ice for 10 min. Centrifuge at 10,000 x g for 20 minutes and transfer the supernatant to a fresh tube. Add 5-50 µl sample per well in a clear 96 well plate & adjust the volume to 50 µl with 6-PGD Assay Buffer. For the 6-PGD positive control, dilute the required amount 10 times with assay buffer, add 2 µl of the diluted 6-PGD Positive Control per well into the desired well(s) and adjust the final volume to 50 µl with 6-PGD Assay Buffer.

   Notes:

   - For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.
   - Residual NADPH in samples will generate a background. This background can be corrected for by making a background control mix (omitting the 6-PGD substrate in the reaction) and running a parallel sample background control.

2. **NADPH Standard Curve:** Add 0, 2, 4, 6, 8 and 10 µl of 1 mM NADPH Standard into a series of wells in 96 well clear plate to generate 0, 2, 4, 6, 8 and 10 nmol/well of NADPH Standard. Adjust the volume to 50 µl/well with 6-PGD Assay Buffer.

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

	Reaction Mix	Background Control Mix
6-PGD Assay Buffer	40 µl	42 µl
6-PGD Developer	8 µl	8 µl
6-PGD Substrate	2 µl	—

Mix and add 50 µl of the Reaction Mix to each well containing the Standard, Positive Control and test samples. For background correction, add 50 µl of Background Control Mix (without substrate) to sample background control well(s) and mix well.

4. **Measurement:** Measure absorbance immediately at 460 nm in kinetic mode for 45-60 min at 37°C.

Note: Measurement time for the linear phase of the reaction depends on the 6-PGD activity in samples. We recommend measuring the absorbance in kinetic mode, and choosing two time points (t_1 and t_2) in the linear range to calculate the 6-PGD activity of the samples. The NADPH Standard Curve can be read in endpoint mode (*i.e.* at the end of the incubation time).

5. **Calculation:** Subtract the 0 nmol Standard reading from all Standard Curve readings. Plot the NADPH Standard Curve. If sample background control reading is significant, subtract the background control reading from its paired sample reading. Calculate $\Delta OD_{460} = A_2 - A_1$ from the linear phase of the test sample reaction. Apply the ΔOD value to the NADPH Standard Curve to get B nmol of NADPH generated during the reaction time ($\Delta T = t_2 - t_1$).

Sample 6-Phosphogluconate Dehydrogenase Activity = B/(Δt X V) x D = nmol/min/ml = mU/ml

Where: **B** = NADPH amount from Standard Curve (nmol).
Δt= reaction time (min).
V = sample volume added into the reaction well (ml).
D = Dilution Factor

Unit Definition: One unit of 6-phosphogluconate dehydrogenase is the amount of enzyme that generates 1.0 µmole of NADPH per min at pH 8.0 at 37°C.

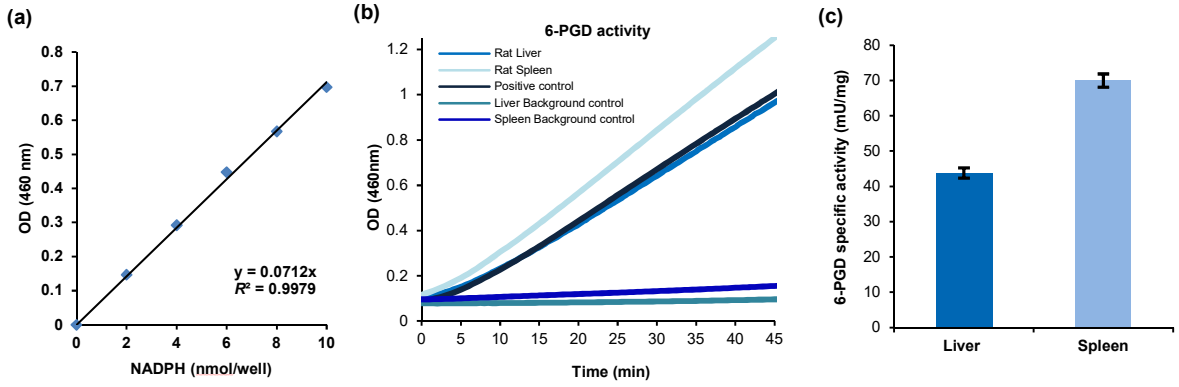


Figure: (a) NADPH standard curve; (b) Reaction kinetics of 6-Phosphogluconate dehydrogenase activity in positive control, rat liver (66 µg protein) and rat spleen (56 µg protein) using appropriate background controls; (c) 6-Phosphogluconate dehydrogenase specific activity calculated in rat liver and spleen tissue lysates. Assays were performed following the kit protocol.

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