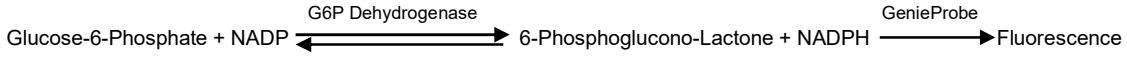
Glucose-6-Phosphate Dehydrogenase Activity Assay Kit (Fluorometric) (#BN00964)

(Catalog #BN00964; 100 assays; Store at -20°C)

I. Introduction:

Glucose-6-Phosphate Dehydrogenase (G6PDH: EC 1.1.1.49) is a cytosolic enzyme in the pentose phosphate pathway, a metabolic pathway that supplies reducing energy to cells (such as erythrocytes) by maintaining the level of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). The NADPH in turn maintains the level of glutathione in these cells that helps protect the red blood cells against oxidative damage. Of greater quantitative importance is the production of NADPH for tissues actively engaged in biosynthesis of fatty acids and/or isoprenoids, such as liver, mammary gland, adipose tissue, and adrenal gland. Assay Genie's Glucose-6-Phosphate Dehydrogenase Assay kit provides a quick and easy method for monitoring G6PDH activity in a wide variety of samples. In this assay, G6PDH converts G6P into pyruvate and NADPH, which further reduces GenieProbe to generate an intense fluorescence product (Ex/Em = 535/587 nm). This kit is simple, sensitive and high-throughput adaptable and can detect as low as 1 µU of G6PDH activity.



II. Applications:

- Measurement of G6PDH activity in various tissues and cells
- Evaluation of pentose phosphate pathway

III. Sample Type:

- Animal tissues: muscle, liver, heart, kidney, etc.
- Cell culture: adherent or suspension cells
- Plant tissues

IV. Kit Contents:

Components	BN00964	Cap Code	Part Number
G6PDH Assay Buffer	25 ml	WM	BN00964-1
GenieProbe (in DMSO)	0.4 ml	Blue	BN00964-2
G6PDH Substrate	1 Vial	Orange	BN00964-3
G6PDH Developer	1 Vial	Red	BN00964-4
G6PDH Positive Control	1 Vial	Green	BN00964-5
NADPH Standard (200 nmol)	1 Vial	Yellow	BN00964-6

V. User Supplied Reagents and Equipment:

- 96-well white 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.

- **G6PDH Assay Buffer:** Bring to room temperature before use. Store at 4°C or -20°C.
- **GenieProbe:** Before use, thaw at room temperature. Store at -20°C. Use within two months.
- **G6PDH Substrate and Developer:** Reconstitute with 220 µl Assay Buffer. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **G6PDH Positive Control:** Reconstitute with 100 µl Assay Buffer and mix thoroughly. Aliquot and store at -70°C. Avoid freeze/thaw. Use within two months. Keep on ice while in use.
- **NADPH Standard:** Reconstitute with 200 µl dH_2O to generate 1 mM (1 nmol/µl) NADPH Standard solution. Aliquot and store at –20°C. Use within two months. Keep on ice while in use.

VII. G6PDH Activity Assay Protocol:

1. **Sample Preparation:** Homogenize tissue (~10 mg) or cells (1 x 10^6) with 100 µl ice cold G6PDH Assay Buffer. Keep on ice for 10 min. Centrifuge at 10,000 X g, 4°C for 5 min. and collect supernatant. Dilute the supernatant ~10 fold in Assay Buffer and add 1-50 µl into desired well(s) in a 96-well plate. For Positive Control, dilute G6PDH Positive Control 200 times with G6PDH Assay Buffer just before use and add 2-20 µl of diluted G6PDH Positive Control into desired well(s). Adjust the volume of Positive Control and sample wells to 50 µl/well with G6PDH Assay Buffer.

   Notes:

   a. For unknown samples, we suggest doing pilot experiment and testing several amounts of G6PDH to ensure the readings are within the Standard Curve range.

   b. If sample has high background, prepare parallel sample well(s) as sample background control.

   c. Don't store the diluted G6PDH Positive Control.

2. **NADPH Standard Curve:** Dilute NADPH Standard to 40 µM (40 pmol/µl) by adding 40 µl of 1 mM NADPH Standard to 960 µl of dH_2O. Add 0, 2, 4, 6, 8, and 10 µl of 40 µM NADH Standard into a series of wells in a 96-well plate to generate 0, 80, 160, 240, 320 and 400 pmol/well of NADPH Standard. Adjust the volume to 50 µl/well with G6PDH 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
G6PDH Assay Buffer	44 µl	46 µl
GenieProbe	2 µl	2 µl
G6PDH Developer	2 µl	2 µl
G6PDH Substrate	2 µl	----

Mix. Add 50 µl of Reaction Mix to each well containing Standards, Positive Control, and samples. Mix well.
* For samples having background, add 50 µl of Background Control Mix to sample background control well(s).

4. **Measurement:** Measure fluorescence (Ex/Em = 535/587 nm) immediately in kinetic mode for 10-40 min. at 37°C.

   Note: Incubation time depends on the G6PDH activity in the samples. We recommend measuring fluorescence in kinetic mode, and choosing two time points (T_1 and T_2) in the linear range to calculate the G6PDH activity of the samples. The NADPH Standard Curve can be read in endpoint mode (i.e. at the end of incubation time).

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the NADPH Standard curve. If sample background control reading is significant, subtract the sample background control reading from sample reading. Calculate the G6PDH activity of the test sample: $\Delta RFU = RFU_2 - RFU_1$. Apply ΔRFU to NADPH Standard Curve to get B pmol of NADPH generated by G6PDH during the reaction time ($\Delta T = T_2 - T_1$).

Sample G6PDH Activity = B/(ΔT X V) x D = pmol/min/µl = µU/µl = mU/ml

Where: **B** is NADPH amount in the sample well from Standard Curve (pmol)
ΔT is reaction time (min.)
V is sample volume added into the reaction well (µl)
D is dilution factor

G6PDH Activity in samples can also be expressed in mU/mg of protein.

Unit Definition: One unit of G6P Dehydrogenase is the amount of enzyme that generates 1.0 µmol of NADPH per min. at pH 8.0 at 37°C.

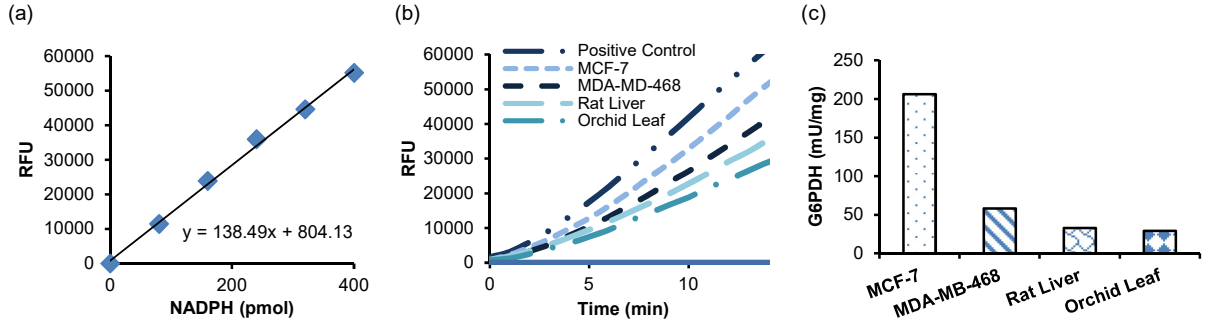


Figure: (a) NADPH Standard Curve. (b) Kinetic measurement of G6PDH activity in various samples. (c) G6PDH specific activity was calculated in lysates prepared from MCF-7 (0.29 µg), MDA-MB-468 (0.41 µg), rat liver (0.6 µg) and orchid leaf (0.56 µg). Assays were performed following the kit protocol.

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