ab303735 – Glucose-6-Phosphate Dehydrogenase Activity Assay Kit (Fluorometric)

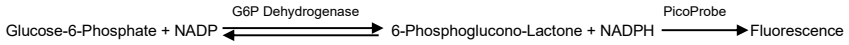
For measuring G6PDH activity in tissue lysate, cell lysate, and plant tissues.
For research use only - not intended for diagnostic use.

Storage and Stability

The entire Assay kit may be stored at -20°C protected from light.

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. The 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 PicoProbe™ 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.



Materials Supplied

Item	Quantity
Assay Buffer 5	25 mL
PicoProbe I	0.4 mL
G6PDH Substrate	1 vial
Developer Mix P	1 vial
G6PDH Enzyme	1 vial
NADPH Standard	1 vial

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

- Upon arrival, store the kit at -20 °C, protected from light.
- Read the entire protocol before performing the assay.

Assay Buffer 5: Bring to room temperature before use. Store at 4°C or -20°C.

PicoProbe I: Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. Keep at room temperature during the assay. Store at -20°C and protect from light and moisture. Once the probe is opened and thawed, it is stable for at least 3 additional freeze/thaw cycles but should be used within two months. After use, promptly retighten the cap to minimize adsorption of airborne moisture.

G6PDH Substrate and Developer Mix P: Reconstitute with 220 µl Assay Buffer 5. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.

G6PDH Enzyme: Reconstitute with 100 µl Assay Buffer 5 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.

Sample Preparation

Homogenize tissue (~10 mg) or cells (1 x 10^6) with 100 µl ice cold Assay Buffer 5. 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 5 and add 1-50 µl into desired well(s) in a 96-well plate. For Positive Control, dilute G6PDH Enzyme 200 times with Assay Buffer 5 just before use and add 5 µl of diluted G6PDH Positive Control into desired well(s). Adjust the volume of Positive Control and sample wells to 50 µl/well with Assay Buffer 5.

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 Enzyme.

Assay Procedure

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 dH2O. 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. Adjust the volume to 50 µl/well with Assay Buffer 5.

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

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer 5	44	46
PicoProbe I	2	2
Developer Mix P	2	2
G6PDH Substrate	2	-

Mix. Add 50 µl of Reaction Mix to each well containing Standards, G6PDH Enzyme (Positive Control), and samples. Mix well.
* For samples having background, add 50 µl of Background Control Mix to sample background control well(s).

2. **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).

Calculations

1. Average the duplicate reading for each standard and sample.
2. Subtract the mean RFU value of the blank (Standard #1) from all standard and sample readings. This is the corrected RFU.
3. Plot the corrected RFU value for each NADPH standard as a function of the final amount (in pmoles).Calculate the linear equation of the standard curve.
4. If sample background control reading is significant, subtract the sample background control reading from sample reading. Find the ΔRFU during the linear range of the sample: $\Delta RFU = RFU_2 - RFU_1$
.
5. Apply ΔRFU to NADPH Standard Curve to get B pmol of NADPH generated by G6PDH during the reaction time ($\Delta T = T_2 - T_1$)
6. Calculate the G6PDH activity of the test sample:

$$Sample\ G6PDH\ Activity = \frac{B}{(\Delta T\ x\ V)} x\ D = \text{pmol}/(\text{min} * \mu\text{l}) = \mu\text{U}/\mu\text{l} = \text{mU}/\text{ml}$$

Where:

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

One Unit is defined as the amount of enzyme that produces 1 µmol of NADPH per minute under the assay conditions.

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

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)

Version 3a | 24-Nov-25