

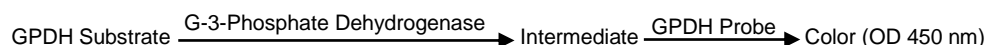
Glycerol-3-Phosphate Dehydrogenase Activity Colorimetric Assay Kit

(Catalog # K640-100; 100 assays; Store at -20°C)

rev. 2/15

I. Introduction:

Glycerol-3-Phosphate Dehydrogenase (EC 1.1.1.8) is an important enzyme for lipid metabolism. It catalyzes the reversible conversion between dihydroxyacetone phosphate and glycerol-3-phosphate. GPDH plays multiple functions inside cells; it links carbohydrate and lipid metabolism, and provides electrons through the Glycerol-3-Phosphate Shuttle. When progenitor adipocytes differentiate into mature adipocytes, GPDH activity increases significantly. Analysis of glycerol-3-phosphate dehydrogenase activity is crucial for the study of fatty acid metabolic pathways. In BioVision's Glycerol-3-Phosphate Dehydrogenase Activity Assay kit, Glycerol-3-phosphate dehydrogenase reacts with the substrate to form an intermediate, which reduces a colorless probe to a colored product with strong absorbance at 450 nm. The assay is simple, sensitive and rapid and can detect Glycerol-3-Phosphate dehydrogenase activity less than 1 mU/well.



II. Application:

- Measurement of Glycerol-3-Phosphate Dehydrogenase activity in various tissues/cells
- Analysis of fatty acid metabolism and cell signaling
- Screening anti-obesity drugs

III. Sample Type:

- Animal tissues: Liver, Brain, Heart, Kidney etc.
- Cell culture: preadipocytes, adipocytes etc.

IV. Kit Contents:

Components	K640-100	Cap Code	Part Number
GPDH Assay Buffer	27 ml	WM	K640-100-1
GPDH Substrate (Lyophilized)	1 vial	Blue	K640-100-2
GPDH Probe (Lyophilized)	1 vial	Red	K640-100-3
NADH Standard (Lyophilized)	1 vial	Yellow	K640-100-4
GPDH Positive Control (Lyophilized)	1 vial	Purple	K640-100-5

V. User Supplied Reagents and Equipment:

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

VI. Storage and Handling:

Store kit at -20°C, protected from light. Warm GPDH Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening.

VII. Reagent Preparation and Storage Conditions:

- **GPDH Substrate:** Reconstitute with 220 µl GPDH Assay Buffer. Store at -20°C. Use within two months. Keep on ice while in use.
- **GPDH Probe:** Reconstitute with 220 µl dH₂O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **NADH Standard:** Reconstitute with 100 µl GPDH Assay buffer to generate 5 mM NADH Standard solution. Store at -20°C. Use within two months. Keep on ice while in use.
- **GPDH Positive Control:** Reconstitute with 100 µl GPDH Assay Buffer and mix thoroughly. Aliquot and store at -20°C.

VIII. Glycerol-3-Phosphate Dehydrogenase Assay Protocol:

1. Sample Preparation: Homogenize tissue (~10 mg) or cells (~1 x 10⁶) with 200 µl ice cold GPDH Assay Buffer for 10 minutes on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. Add 1-50 µl sample per well, adjust final volume to 50 µl with GPDH Assay Buffer. Prepare a parallel sample well as the background control to avoid interference from NADH in the sample. For positive control, take 1-10 µl of reconstituted GPDH Positive Control & adjust the final volume to 50 µl with GPDH Assay Buffer.

Note:

For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

2. NADH Standard Curve: Dilute NADH Standard to 1mM by adding 20 µl of NADH to 80 µl GPDH Assay buffer. Add 0, 2.5, 5, 7.5, 10 and 12.5 µl of diluted 1 mM NADH Standard into a series of wells in 96 well plate to generate 0, 2.5, 5.0, 7.5, 10 and 12.5 nmol/well of NADH Standard. Adjust volume to 50 µl/well with GPDH 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
GPDH Assay Buffer	46 µl	48 µl
GPDH Probe	2 µl	2 µl
GPDH Substrate	2 µl	----

Add 50 μ l of the Reaction Mix to each well containing the Standard, Positive Control and test samples and 50 μ l of Background Control Mix to well(s) containing the background control sample. Mix well.

4. Measurement: Incubate for 20-60 min at 37°C and measure OD at 450 nm.

Note: Incubation time depends on the Glycerol-3-Phosphate Dehydrogenase activity in the samples. We recommend measuring the OD in a kinetic mode, and choose two time points (T_1 & T_2) in the linear range to calculate the GPDH activity of the samples. The NADH Standard Curve can be read in endpoint mode (i.e., at the end of incubation time).

5. Calculation: Subtract the 0 Standard reading from all Standard readings. Plot the NADH Standard Curve. Correct sample background by subtracting the value derived from the background control from sample readings. Calculate the GPDH activity of the test sample: $\Delta OD = A_2 - A_1$. Apply the ΔOD to the NADH Standard Curve to get B nmol of NADH generated by Glycerol-3-Phosphate Dehydrogenase during the reaction time ($\Delta T = T_2 - T_1$).

$$\text{Sample Glycerol-3-Phosphate dehydrogenase activity} = \frac{B}{(\Delta T \times V)} \times \text{Dilution Factor} = \text{nmol/min/ml} = \text{mU/ml}$$

Where: **B** = NADH amount from Standard Curve (nmol).

ΔT = reaction time (min).

V = sample volume added into the reaction well (ml).

Unit Definition: One unit of Glycerol-3-Phosphate Dehydrogenase is the amount of enzyme that will generate 1.0 μ mol of NADH per min. at pH 8 at 37°C.

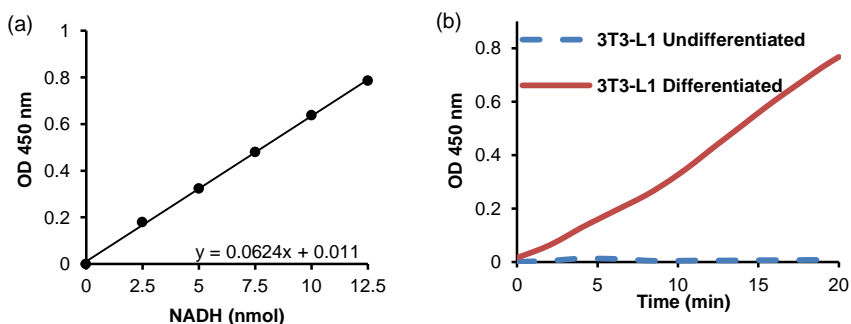


Figure: (a) NADH standard curve. (b) Measurement of Glycerol-3-Phosphate Dehydrogenase activity in 3T3-L1 pre-adipocyte (60 μ g) and differentiated 3T3-L1 adipocytes (60 μ g). Assays were performed following kit protocol.

IX. RELATED PRODUCTS:

Free Glycerol Colorimetric/Fluorometric Assay Kit

Triglyceride Quantification Colorimetric/Fluorometric Kit

Glucose and Sucrose Assay Kit

Glucose Uptake Colorimetric Assay Kit

Glucose Uptake Fluorometric Assay Kit

Maltose and Glucose Assay Kit

Glucose-1-Phosphate Assay Kit

PicoProbe™ Glucose-6-Phosphate Assay Kit

Phosphoglucomutase Assay Kit

Free Glycerol Colorimetric Assay Kit

PicoProbe™ Triglyceride Fluorometric Assay Kit

NAD/NADH Quantification Kit

Glucose-6-Phosphate Dehydrogenase Assay Kit

Glucose Assay kit

NADP/NADPH Quantification Kit

Hexokinase Assay Kit

Glucose Dehydrogenase Activity Assay Kit

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