Glucose Dehydrogenase (GDH) Assay Kit (Colorimetric)

LS-K274-100 (100 Tests) • **Store at -20°C**

LSBio
LifeSpan BioSciences, Inc.

Introduction

GLUCOSE DEHYDROGENASE (GDH) belongs to the family of oxioreductases, specifically those acting on the CH-OH group of donor with other acceptors. GDH participates in the pentose phosphate pathway. This non-radioactive, colorimetric GDH assay is based on the reduction of the tetrazolium salt MTT in a NADH-coupled enzymatic reaction to a reduced form of MTT which exhibits an absorption maximum at 565 nm. The increase in absorbance at 565 nm is proportional to the enzyme activity.

Key Features

- Fast and sensitive. Linear detection range (20 µL sample): 0.5 to 200 U/L for 15 min reaction.
- Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

Applications

- GDH activity determination in biological samples (e.g. plasma, serum, tissue and culture media.)

Components

Component	K274-100
	100 Tests
Assay Buffer	10 mL
NAD/MTT	1 mL
Substrate	1 mL
Diaphorase	120 µL
Calibrator	1.5 mL

Materials Not Supplied

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and plate reader.

Storage

The kit is shipped at ambient temperature. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

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Assay Procedure

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Assays can be executed at any desired temperature (e.g. 25°C or 37°C).

Sample Preparation

Serum and plasma are assayed directly.

Tissue: prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) in ~200 µL buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 × g for 15 min at 4°C. Remove supernatant for assay.

Cell Lysate: collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 × g for 15 min at 4°C. Remove supernatant for assay.

All samples can be stored at –20 to –80°C for at least one month.

Reagent Preparation

Equilibrate reagents to desired reaction temperature (e.g. 25°C or 37°C). Briefly centrifuge tubes before use. Prepare enough Working Reagent (WR) for all assay wells by mixing, for each 96-well assay: 8 µL Substrate, 8 µL NAD/MTT Solution, 1 µL Diaphorase and 70 µL Assay Buffer.

Procedure

1. Transfer 100 µL H_2O (OD_{H2O}) and 100 µL Calibrator (OD_{CAL}) solution into wells of a clear flat bottom 96-well plate.
2. Transfer 20 µL of each sample into separate wells and then add 80 µL WR to each sample well. Tap plate briefly to mix.
3. Read OD_{565nm} (OD_0), and again after 15 min (OD_{15}) on a plate reader.

Calculations

Subtract the OD_0 from OD_{15} for each sample to compute the ΔOD_S values. GDH activity can then be calculated as follows:

$$\text{GDH Activity} = \frac{\Delta OD_S}{\varepsilon_{mtt} \cdot l} \times \frac{\text{Reaction Vol (µL)}}{t\,\text{(min)} \cdot \text{Sample Vol (µL)}} \times n$$

$$= \frac{\Delta OD_S}{OD_{CAL} - OD_{H20}} \times \frac{273}{t\,\text{(min)}} \times n \quad \textit{(U/L)}$$

where ε_{mtt} is the molar absorption coefficient of reduced MTT. l is the light path length which is calculated from the calibrator. OD_{CAL} and OD_{H20} are OD_{565nm} (OD_o) values of the Calibrator and water. t is the reaction time (15 min is the recommended time). Reaction Vol and Sample Vol are 100 µL and 20 µL, respectively. n is the dilution factor.

Unit definition: 1 Unit (U) of GDH will catalyze the conversion of 1 µmole of NAD to NADH per min at pH 8.2.

Note: If sample GDH activity exceeds 200 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. For samples with GDH activity < 5 U/L, the incubation time can be extended up to 2 hours.

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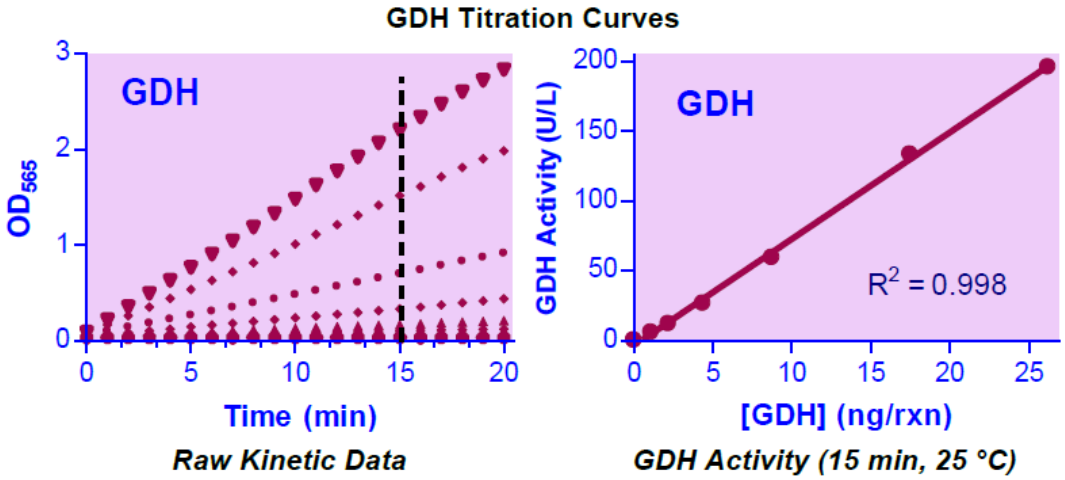
Sample Data



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