Technical Bulletin

Glucose Dehydrogenase Assay Kit

Catalog Number MAK450

Product Description

Glucose Dehydrogenase (GDH) belongs to the family of oxidoreductases. Oxidoreductases are unable to utilize oxygen as the electron acceptor and instead transfer electrons to various natural and artificial electron acceptors. GDH participates in the pentose phosphate pathway.

The Glucose Dehydrogenase Activity Assay Kit is a non-radioactive, colorimetric GDH assay. The method 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. The linear detection range is 0.5 to 200 units/liter (U/L) for a 20 µL sample in a 15 minute reaction.

The kit is suitable for the quantitative determination of D-glucose dehydrogenase enzyme activity and drug effects on its metabolism in biological samples (e.g., plasma, serum, tissue and culture media).

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalog Number MAK450A
- Diaphorase 120 µL
  Catalog Number MAK450B
- NAD/MTT 1 mL
  Catalog Number MAK450C
- Calibrator 1.5 mL
  Catalog Number MAK450D
- Substrate 1 mL
  Catalog Number MAK450E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- Microcentrifuge capable of RCF ≥ 10,000 × *g*
- 1.5 mL microcentrifuge tubes
- Phosphate Buffered Saline (PBS) (Catalog Number P3813 or equivalent)
- Potassium phosphate monobasic (Catalog Number P0662 or equivalent)

Precautions and Disclaimer

For R&D Use Only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Procedure

All samples and standards should be run in duplicate.

Assays can be executed at any desired temperature (e.g., 25 °C or 37 °C).

Sample Preparation

Serum and plasma

Serum and plasma samples can be assayed directly.

Tissue

1. Prior to dissection, rinse tissue in phosphate buffered saline, pH 7.4, to remove blood.
2. Homogenize tissue (50 mg) in 200 µL of buffer containing 50 mM potassium phosphate, pH 7.5.
3. Centrifuge at 10,000 × *g* for 15 minutes at 4 °C.
4. Remove supernatant and retain for assay.

Cell Lysate

1. Collect cells by centrifugation at 2,000 × *g* for 5 minutes at 4 °C.
2. For adherent cells, do not harvest cells using proteolytic enzymes. Instead, use a rubber policeman or cell scraper.
3. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate, pH 7.5.
4. Centrifuge at 10,000 × *g* for 15 minutes at 4 °C.
5. Remove supernatant and retain for assay.

All samples

1. For unknown samples, test several dilutions to ensure that the readings are within the linear range of the Standard Curve.
2. All samples can be stored at -20 °C to -80 °C for at least one month.
3. Transfer 20 µL of each Sample to separate wells of a clear 96-well plate.

Working Reagent

Mix enough reagents for the number of assays to be performed. For each well, prepare 87 µL of Working Reagent according to Table 1.

Table 1.
Preparation of Working Reagent

Reagent	**Working Reagent**
Assay Buffer	70 µL
Substrate	8 µL
NAD/MTT	8 µL
Diaphorase	1 µL

Assay Reaction

Note: 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.

1. Transfer 100 µL of purified water (OD_{H2O}) and 100 µL of Calibrator (OD_{CAL}) into separate wells of the plate.
2. Add 80 µL of Working Reagent to each **Sample** well. **Do not** add Working Reagent to Water or Calibrator wells.
3. Tap plate briefly to mix.

Measurement

Read optical density (OD) at 565 nm immediately (Time Zero, OD_0), and again after 15 minutes (OD_{15}) on a plate reader. The Water Blank (OD_{H2O}) and Calibrator (OD_{CAL}) can be read at the end of the incubation time.

Results

1. Calculate the corrected Sample optical density values (ΔOD_S) by subtracting the OD_0 value from OD_{15} for each Sample.
2. Calculate GDH activity as follows:

GDH (U/L) =

$$\frac{\Delta OD_S \times RxnVol\ (\mu L) \times DF}{\varepsilon_{MTT} \times L \times T \times SmplVol\ (\mu L)}$$

=

$$\frac{\Delta OD_S \times 273 \times DF}{(OD_{CAL} - OD_{H2O}) \times T}$$

where

ΔOD_S = Sample OD_{15} value minus OD_0 value

OD_{CAL} = OD value at 565 nm of Calibrator

OD_{H2O} = OD value at 565 nm of the Water Blank

RxnVol = Final reaction volume (100 µL)

T = Reaction time (standard procedure is for 15 minutes but may be extended or shortened due to low or high activity)

SmplVol = The amount of Sample (µL) used in the reaction (20 µL)

ε_{MTT} = Absorptivity coefficient of reduced MTT

L = Light pathlength in cm. The light path for the 96-well assay is calculated from the Calibrator optical density.

DF = Sample Dilution Factor (DF = 1 for undiluted Samples)

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

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

Figure 1.
GDH titration curve, raw kinetic data.

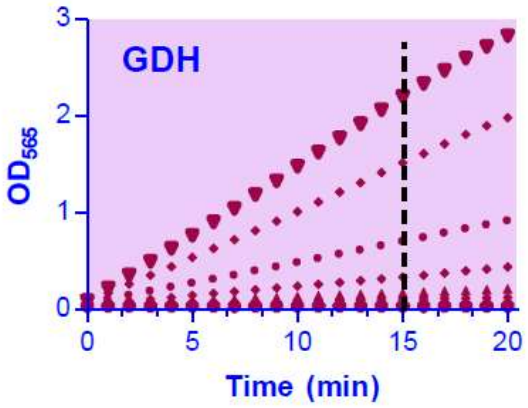
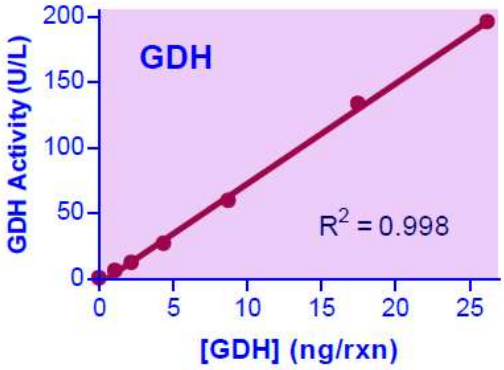


Figure 2.
GDH titration curve. GDH Activity (15 minutes at 25 °C).



References

1. Bak, T.G., Studies on glucose dehydrogenase of *Aspergillus oryzae*. II Purification and physical and chemical properties. *Biochim. Biophys. Acta*, **139**, 277–93 (1967).
2. Thompson, R.E., and Carper, W.R., Glucose dehydrogenase from pig liver. I. Isolation and purification. *Biochim. Biophys. Acta*, **198(3)**, 397–406 (1970).

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